AVITAMINOSES A AND/OR E RABBITS ON SEMI-PURIFIED DIET: ALTERATIONS IN BLOOD, URINE, TISSUE CONSTITUENTS, AND CELLULAR ACTIVITY

by

KONGARA SATYANARAYANA RAO

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INTRODUCTION

Although deficiency diseases resulting from the lack or nonavailability of essential nutrients have been recognized for a long time, the exact mechanism for manifestation of the concommitant symptoms, tissue changes, and tissue constituents still remains a mystery in most cases. This aspect of study has interested many an investigator who endeavored to unravel the intricate mechanisms of deficiency syndromes and correlate them with the role these nutrients play in cellular processes.

Even though it has been possible to create a single deficiency of a particular nutrient experimentally, either in large or small laboratory animals, most deficiencies occur in nature as a result of multiple nutrient deficiencies. The interplay between them, whether synergistic or antagonistic, is yet a subject of much interested study and controversy.

In an experimentally produced deficiency, most of the functional and anatomical changes which are associated with the lack of an exogenous dietary essential have been described sufficiently. However, it is possible that certain cells and tissues of the organism may suffer from the lack of an endogenous nutrient or metabolite that is ordinarily made from exogenous material. Certain vitamins are known to take part in enzyme systems or in the synthesis and release of hormones. To what extent the changes in tissues are uncomplicated by the deficiency of these hormones is not yet known. Any deficiency may elicit a stress response which may complicate the simple deficiency state either created or naturally occurring. Stress

caused by environmental factors has also been accredited with precipitating latent manifestations of deficiency diseases.

Sufficient proofs or data are not available, in most instances, with which to ascribe a particular biochemical lesion as being produced purely by the deficiency of any one essential nutrient, since similar changes have been known to be produced by the lack of other dietary essentials. The normal interplay or association in biological function between such functionally related nutrients is a subject of much active study.

Studies concerning multiple nutrient deficiencies are of great importance, not only to throw light on the naturally occurring endemic deficiencies, but also to help understand the biological synergism or antagonism between such nutrients.

Although a great amount of knowledge has been accumulated regarding avitaminoses A and E, still much needs to be done to unravel the biological role, their mutual dependence, and the functional interrelationship of these vitamins.

In avitaminosis E, the tissue vitamin A levels are rapidly depleted (161, 45). That vitamin E protects vitamin A from oxidative destruction has been shown (46). Previous work at this laboratory has indicated, though not conclusively proved, that two vitamins, A and E, are in some way concerned with the function of adrenal cortex. Vitamin A has been reported to be actively involved in biosynthesis of glucocorticoids (138, 256, 257, 258). Hypertrophy of the adrenal cortex has been recorded in vitamin E deficiency syndromes (22, 23, 108). To what extent these two vitamins are interrelated in the biogenesis of adrenal

corticoid hormones is a question as yet unanswered.

Recent studies on the etiology of muscular dystrophy in domestic animals have shown that vitamin E is not the only factor involved, but that selenium, which has now assumed the status of an essential nutrient, is also incriminated, at least in some species (62, 66, 173, 180, 213). The rate of the dystrophic muscle respiration has been reported to be higher (81, 111, 113, 119, 131, 207, 246).

Varying results have been reported regarding the changes in tissue ubiquinone concentrations in avitaminosis E. No indication was found of a change in ubiquinone levels in tiasues of vitamin E deprived animals (161, 170), but other workers (61, 62, 91) reported a decrease in tissue ubiquinone levels.

With these factors in mind a project to investigate the physiological effects of avitaminoses A and/or E and attempt to determine the intricate relationship between these two vitamins was therefore instituted.

PURPOSE OF STUDY

The present experiments were deaigned to study the following relationships:

(a) The biological role of vitsmins A snd E in the synthesis of adrenal corticosteroids;

(b) The effects of avitaminoses A and/or E on sodium, potassium, calcium, and magnesium metabolisms;

(c) The role of vitamins A and/or E in ubiquinone biosynthesis;

(d) The effect of selenium on cell respiration in avitaminoses E and/or A;

(e) The changes taking place in the blood and urine as the deprivation state progresses towards termination.

REVIEW OF LITERATURE

Historical

As reported by Blaxter (17), the earliest observation of nutritional muscular dystrophy was made in 1854, although the etiology of the disease was not known at that time. The condition, reported in calves in 1866, was thought to be caused by "Sarkosporidien," and was named accordingly as "Huhnerfleisch." On observing the apparent changes in the muscles of affected calves and swine, this condition was later called "Fischfleish" in 1898. The following year, the condition described as "Wachsartige", degeneration in the skeletal muscles of calves, sheep, and swine, was reported. In another syndroms "Weissenfleish" in six to seven-week-old calves, the histopathological changes were considered to be a reversal of embryonic development in striated muscle (17). Based on their observations of scorbutic changes in the teeth and skeletons of affected calves, Hjarre and Lillesngen (110) attributed naturally occurring muscular dystrophies of calves to vitamin C deficiency. That cod liver oil was responsible for the causation of lesions was expressed in many of the reports of Agduhr (2); these reports stimulated considerable work on other animals. He claimed himself to be

the first to make such observations as early as 1922, although the earliest description was reported by Slagvold in 1925 (221).

With the discovery of vitamin E's role in fetal resorptions in rats by Evans and Bishop in 1922 and 1923 (71), this new factor has come to be known as the antisterility factor. The chemical nature of vitamin E was worked out by Emersone and Evans et al. (71), who later named this substance "tocopherol."

The so-called stiff lamb disease was first reported in the United States by Metzger and Hogan (156), who concluded that the disease was a queer degenerative process beginning with the actual contractile substance of the voluntary muscalature.

The experimental production of nutritional muscular dystrophy in rabbits and encephalomalacia in chicken was first demonstrated by Goettsch and Pappenheimer (85) in 1930 and 1931. In their monumental work they were able to show that the elimination of vitamin E from the diet was a factor in the production of muscular dystrophy. Similar dystrophies due to avitaminosis E were reported in mice by Pappenheimer in 1942 (185); in rats by Knoulton and Hines (132); in chicks by Pappenheimer (187); in ducks by Victor (246); in dogs by Anderson, Elvehjem, and Gonce in 1939 (4); in monkeys by Mason and Telford in 1947 (152); and in cattle by Gullickson and Calverly in 1946 (95). In 1947 Vawter and Records (243) discovered a number of farms in Nevada, U.S.A., in which calves on range died with symptoms of muscular dystrophy comparable to those associated with stiff lamb disease as described by Willman et al. (253), a disease known to be cured by DL-alpha-tocopherol acetate. In cattle, the studies of Gullikson

and Calverly (96) and Gullikson et al. (95) revealed that vitamin E-deficiency was associated with sudden death resulting from cardiac failure and a decreased functional activity of the myocardium as evidenced by electrocardiograms.

The deficiency of vitamin E manifests itself in a variety of ways, culminating in the production of different types of syndromes associated with varied symptoms and lesions. A species variation has been observed in the way which certain symptoms or lesions are predominantly manifested.

Based on the above observations, Diplock et al. (61) have classified the vitamin E-deficiency syndromes into four major types:

(1) Steatopathies, which include yellow fat disease of mink, fox, ferret, cat, and pig; the least complicated condition more or less due to the dearth of antioxidants and vitamin E.

(2) Liver diseases, which include necrotic liver degeneration in mouse and rat, and hepatitis dietetica in the pig, produced by a combined deficiency of alpha-tocopherol and selenium, which can be prevented by either.

(3) A combined deficiency of selenium and vitamin E, although apparently unrelated physiologically and diverse in their nature, is caused by and can usually be completely prevented by the presence of one of these nutrients but only partially by the other. In several diseases of this group, lipid peroxidation may play a

subsidiary part and can be exacerbated by the presence of unsaturated fat. These diseases can cover a range in which the requirements of selenium and alpha-tocopherol appear to be mutually dependent. On the one extreme "exudative diathesis" of chicks which responds completely to alpha-tocopherol but not quite to selenium, and at the other extreme "stiff lamb disease" which is prevented by selenium but with difficulty by alpha-tocopherol, requiring large doses.

(4) A group of diseases apparently prevented by vitamin E--many conditions effecting reproduction and embryonic development, testicular degeneration, encephalomalacia of chick, muscular dystrophy of rabbit, rat and pig, and diminished resistance of erythrocytes to hemolysis in several species.

Vitamin E-deficiency syndromes are known to occur in many animal species, and have been produced experimentally in the same animal species. However, the naturally occurring syndromes are a little different from those experimentally produced, which is suggestive of some other factors, as yet unknown, being involved. Such great diversity of syndromes which occur in different species would cause doubt in one's mind as to whether or not all such syndromes are pure uncomplicated deficiencies of vitamin E. These observations also emphasize the between species variations for which no satisfactory explanation has yet been put forth.

Muscular dystrophy has been recorded in well over 20 species of animals including camels, buffaloes, kangaroos, and

quokkas (15).

Vitamin E-Deficiency in Rabbits

Rabbits are one of the most commonly used laboratory animals for investigation of vitamin E-deficiency because of the very severe muscular dystrophy shown after a relatively short period on deficient dietary regimens. However, relatively little is known about the mechanism whereby such profound structural alterations in the muscles are produced.

Experimentally, muscular dystrophy has been produced by feeding a semi-purified diet lacking in vitamin E or by using natural diets apparently deficient in tocopherols, with the addition of cod liver oil or other unsaturated fats or fatty acids. Goettsch and Pappenheimer (85) were among the first investigators to produce avitaminosis E in rabbits using a diet containing cod liver oil. MacKenzie et al. (145) and Borgman (21) studied the differencea in the development of avitaminosis E, with and without the addition of cod liver oil. Other workera in the field, like Morgulis and Spencer (167) and Hove et al. (117), used cod liver oil as a stressor.

Three stages have been recognized by MacKenzie and McCollum (144) who made some intensive study of nutritional muscular dystrophy in rabbits. The first was characterized by increased urinary creatine excretion from 18 days before to one day after attainment of maximum weight. The urinary creatinine was unaffected. The average duration was seven days after the maximum weight had been reached. During the second stage physical

symptoms become apparent with the forelegs held stiff and head slightly retracted. Righting of posture when placed on their sides becomes slow. Creatine excretion increases further, food intake decreases, and body weight loss was accelerated. This stage lasted about two to six days. In the third stage, which may occupy one to four days, the creatine excretion was high and decline in weight was rapid. Rabbits placed on their sides regained their upright position only after a violent struggle or remained prostrate for several days before death. Tonus of the skeletal muscles was lost. The most accurate indication of the onset of dystrophy was the increase in creatine excretion, while cessation of growth occurred at the same time. However, the rate of the deprivation for each stage varied with age of the rabbits at the time of placing them on the dietary regimen, the tissue reserves, and environmental or external stresses to which the animals were subjected during the experiment. The order of development of symptoms was the same.

Zalkin (263), who used three per cent cod liver oil in the diet, reported that the rise of urinary creatine levels and the appearance of earliest recognizable histopathological lesions in the muscles was observed after ten days on the vitamin E-deficient diet; and a very marked increase in urinary amino acid excretion commenced five days after that. Gross histopathological lesions and characteristic clinical signs of muscular dystrophy became evident on the seventeenth day. His criterion for the diagnosis of muscular dystrophy was the inability of the animals to right themselves when placed on their sides.

Hove et al. (117) produced acute muscular dystrophy within three weeks by feeding four-week-old male rabbits a diet containing soyabean meal, vitamin-free lard, and cod liver oil. Four grades in the development of muscular dystrophy were described and based on the observation of physical symptoms. Typical duck waddle was considered as grade one; inability to right itself when placed on its side as grade two; inability to remain upright when placed upright as grade three; and the terminal stages as grade four. The last two grades are probably equivalent to stage three described by MacKenzie and McCollum (144).

Histopathological Changes

The affected muscles appear pale with tangible hemorrhagic streaks intermingled with minute pale spots and large areas of necrosis. In severe cases the muscle becomes friable with patches of yellow necrotic tissue (122).

Areas of degenerated skeletal muscle stain darker, fibers appear broken, the ends of myofibrils were frayed, and muscle cells lose their striations. However, not all muscle cells are affected equally; many appear normal with the adjacent ones showing severe degeneration. Interstitial edema, though slight, followed by swelling and hyaline or Zenker's degeneration was noticed. Some cells appeared as if only the nuclei of sarcolemma had been left. Coagulative necrosis with vacuole formation occurred and was finally replaced by connective tissue (16, 21, 85, 122, 143, 144, 184, 185). Perivascular infiltration of

of round cells in muscles was not only noticed in rabbits, but also in guinea pigs, chickens, and ducklings (85).

In some cases a greater part of the muscle was replaced by fibrous connective tissue containing occasional muscle fibers with well preserved cross striations (145). Borgman (21) reported that lesions did not appear in rabbits on a semi-purified diet without cod liver oil until about the fourth week, becoming moderately severe on about the fifth week; whereas in the cod liver oil supplemented group the lesions were similar but more severe.

Innes and Yevich (122) reported that cardiac muscle contained areas of degeneration. Myocardium sometimes showed localized foci of degeneration and increased nucleation resembling that found in the skeletal muscle. However, the extent of degeneration was much less and hyalinization was not usually noticed, the lesions appearing as coagulative necrosis (21, 24, 82, 83, 116). Myocardial degeneration with increased nucleation occurred only in a few cases on semi-purified diets, whereas in the cod liver oil supplemented animals myocardial degeneration was comparatively greater (21).

MacKenzie (143) and Pappenheimer (184) did not observe any interference with reproduction or testicular degeneration in vitamin E-deficient rabbits. On the contrary, testicular degeneration with giant cell formation was reported by Underbjerg (240) employing rabbits maintained for long periods on a vitamin E-deficient diet. Borgman (21) reported slight to moderate loss of spermatocytes and presence of some multinucleated cells

having two or three nuclei.

Irregularities indiceting abnormalities in cardiac muscle metaboliem and transmieeional defects due to degenerative changes have been recorded in electrocardiograms of rabbits with avitaminosis E. Decreaeed heart rate, increaeed TP and QT intervals, elevated ST segments and inverted T waves, changes in the electrical axis of the heart ae evidenced by alteration of potentials of QRS complex, and reduction in the potentials of P and T waves were observed by Martin and Faust (149), Bragdon and Levine (24), Mulder et al. (172), and Gatz and Houchin (82, 83). Borgman (21) believed that the alterations in electrocardiogram ehowing increased frequency of T wave inversions in leed III, right axie deviatione, snd lengthening of QRS and QT intervale were due to mild changes in myocardium with poeeible lesions and functional changes of a diffuse neture in the myocardium.

Victor (246) reported that the creetine content of the dystrophic muscle was lower. Later, Morgulis and Spencer (167) noticed an increased output of creatine in urine, which was considered as the most accurate indication of muscular dystrophy oneet. These observatione were later confirmed by MacKenzie and McCollum (144). Creatine-creatinine ratio increased with the progress of myopathy in rabbits fed tocopherol deficient diet, as reported by Draper (63) and Borgman (21), who have taken this ratio as an indication of oneet of dyetrophic changes in the muscle.

Heinrich and Mattil (104) stated that muscle creatine content decreased, and the rate of creatine synthesis and turnover

ratio were elevated. The work of Melville and Hummel (155) revealed that the glycocyamine synthesis was not affected. They demonstrated that creatinuria and loss of muscle creatine precede the external signs of paralysis and any observable histological changes, and that the level of creatine in liver, kidney, and blood increased in the vitamin E-deficient rabbit. An increased synthesis and turnover of liver creatine was suggested by the work of Roche and his co-workers (206) who reported significantly higher incorporation of isotopic nitrogen into creatine in a case of progressive muscular dystrophy. Confirming the earlier work of Dinning (54, 55), which showed that the creatine was formed and excreted rapidly from the muscles, Dinning and Fitch (59) studied in detail the creatine metabolism of rabbits in vitamin E-deficiency by injecting glycine 1 - C^{14}. Elevated concentration of liver creatine and reduced concentration of skeletal muscle creatine was reported (59). However, kidney glycocyamine or heart muscle creatine was not altered. This was contrary to the earlier report of Mulder *et al.* (172) who recorded a reduction in creatine content of the heart muscle. The turnover rate for heart muscle and skeletal muscle creatine was greater than normal. It was presumed that the creatinuria in avitaminosis E was due in part to the inability of skeletal muscle to retain creatine after its incorporation, since the phosphorylation of creatine was not impaired. Brown and Dyer (28) reported elevated levels of blood creatine and stated that these high levels of creatine may be taken as an indication of muscular dystrophy. Recently Zuckerman and Marquardt (264) recorded low muscle

creatine levels in nutritional muscular dystrophy of rabbits.

Glycogen, Inorganic Phoaphate, and Adenosine Phoaphate Levels

Cohen and Warringa (37) concluded from their findings that the inorganic phosphates, glycogen and adenosine phosphatea remained pretty much the same in skeletal muscle and myocardium of vitamin E-deficient rats. However, the radioactive phoaphoroua turnover was greater in the skeletal muscles. Theae results were confirmed by Fitch and Dinning (77). Contrary to the above-mentioned findings, Calvert et al. (31) demonstrated increased total and inorganic phosphoroua of dystrophic muscles, compared to normal controls, and they opined that this increase was due to a general increase in the permeability of the muacle cell rather than to a specific increase in turnover ratea. Fitch and Dinning (77) conclusively demonstrated that there was a decreased turnover of adenosine triphosphate and creatine phoaphate in vitamin E-deficiency.

Changes in Protein Metabolism

During the course of muscular dyatrophy proteolytic activity of the skeletal muscle was greater in rabbits deficient in vitamin E. At least two proteolytic enzymea were described, one having an optimal pH of 3.8 to 4.0 and the other pH 9.0 (249).

In avitaminoais E rabbits, Dinning (56) obaerved increased excretion of free amino acids in urine, which became apparent

before any physical signe of dyetrophy were noticed. These observatione were confirmed later by Diehl (50) who aleo reported an exceptional behavior in glycine excretion, which decreaeed elightly or remained the same. Thie obeervation gave rise to the belief that glycine was implicated in the metabolism of the dystrophic muscle. Tallen (236) and Smith and Nelson (203) recorded increased concentrations of most of the free amino acids except glycine in the muscle extracts of dyetrophic rabbits. Addition of dietary glycine to dystrophic rabbits prevented the reduction of tissue phoephatase (224). Vitamin E-deficient rabbite incorporated four times more radioactivity after injection of glycine 1 - C^{14} than the normal rabbite (56). Injected glycine was incorporated in all sub-cellular fractions uniformly in vitamin E-deficient rabbits (52). Diehl (50) reported that labelled lysine and leucine were not increased in muscles and explained this ae being due to a higher epecific activity of glycine in skeletal muscle protein synthesie or due to any increaeed interconversion of glycine to other amino acids or other eubetancee like creatine or purine ring bioeyntheeis for nucleic acid production.

Muscle homogenates from vitamin E-deficient rabbits had high values of ribonuclease, cathepsin, beta galactosidase and arylsulfatase enzymes aseociated with lysozymes. In a time couree etudy the increase of these enzymes was cloeely correlated with hietopathological changes and biochemical changes of vitamin E-deficiency (263).

Apart from alteration of muscle protein metabolism, changes

in the plasma protein pattern were reported by many workers (7, 181). Oppenheimer and Milman (181) specifically demonstrated a significant reduction in albumin:globulin ratio with a considerably increased relative concentration of beta-globulin. This increased beta-globulin reflected an increase of lipoproteins, since there was a corresponding increase in the beta-lipid band in the electrophoretic fractionation. That these changes could be attributed to the vitamin E-deficiency was proved by the restoration of the electrophoretic pattern of plasma proteins to normal and the disappearance of the clinical symptoms when the vitamin was administered to dystrophic animals. Similar changes in the plasma proteins were reported in lambs by Baldev (7).

A marked decline in myoglobin concentration was demonstrated in the muscles of guinea pigs when maintained for a long period on a vitamin E-deficient diet. Supplementation with vitamin E prevented the decline in myoglobin (211). The fall in myoglobin level in dystrophic muscles was prevented by weekly administration of diphenyl-p-phenylenediamine, an antioxidant (29).

Changes in Nucleic Acid Metabolism

Increased numbers of nuclei in rabbit skeletal muscle (122, 143, 185), of giant multinucleated cells in rat (151) and rabbit testes (217), and many erythroid precursors in the bone marrow of the monkey (194) in avitaminosis E suggested that vitamin E probably has a role in nucleic acid and nucleoprotein biosynthesis. Dinning (54) and Dinning and his co-workers (60)

found increased incorporation of formate - C^{14} into nucleic acids of liver and skeletal muecle from vitamin E-deficient rats and rabbits. Dinning (58) reported a great enhanced concentration of desoxy ribonucleic acid per gram wet weight of skeletal muscle in avitaminoeie E employing rabbits and monkeye. Treatment with vitamin E produced a eignificant increase in both ribonucleic acid and desoxy ribonucleic acid in teetes, skin, and liver, but only ribonucleic acid in skeletal muscle of rats (125). These findinge indicated an increased turnover of nucleic acids in the skeletal muscle of dystrophic animals, probably as a compensatory measure for loss of protein from the muscle fibers.

Changes in Tiseue Reepiration and Metabolism

A marked increase in oxygen coneumption of muscle etrips and tissue hemogenatee from vitamin E-deficient rate, rabbits, hamsters, and guinea pige was observed by many workers (81, 112, 113, 114, 119, 120, 207, 246). An inverse relationship between creatine content and <u>in vitro</u> oxygen consumption of dystrophic muscle was reported by Victor (246). However, Friedman and Mattil (81) demonstrated that the Q o_2 values for dystrophic muecles of rabbits, rats, and guinea pigs were eignificantly higher than the normal controls. Kawnitz and Pappenheimer (131); Houchin (112), Friedman and Mattil (81); Houchin and Mattil (113, 114, 115); and Hummel and Basinski (119), working with dyetrophic chicken and rat muecles, also recorded an increased oxygen uptake <u>in vitro</u>.

Since the Q o_2 values of dystrophic muscles of diverse animal species were found to be elevated, the effect of administration of tocopherol to the dystrophic animal before the muscle preparation was made, or in vitro addition of tocopherol to the tissue preparation at the time of oxygen uptake study was investigated. Houchin (112, 113) demonstrated that within 27 hours after administration of alpha-tocopheryl acetate, the Q o_2 values were surprisingly reduced to normal without significant regeneration of normal muscle as indicated by persistent high chloride content. The in vitro addition of a water-soluble preparation of alpha-tocopheryl phosphate to the muscle strip or slice was shown by Houchin (112) and Houchin and Mattil (113) to be effective in reducing oxygen consumption of dystrophic muscles of rabbits and hamsters.

Experiments using rabbit tissues from animals raised on stock diets with or without the addition of tocopherol revealed that the oxygen uptake of deficient animals' muscles was 220 per cent higher than the tocopherol supplemented ones, which helped elucidate the fact that tocopherols have an important function in muscle metabolism.

Comparing oxygen consumption of muscle strips with muscle slices, Houchin and Mattil (113) concluded that while muscle strips gave higher values, dystrophic muscle slices showed only slight differences when compared with normal muscles. Hummel and Melville (120), who made similar comparisons, confirmed the earlier findings. Friedman and Mattil (81), while studying the metabolism of the dystrophic muscle at various stages reported

that oxygen uptake was abnormally rapid during the incipient etage when the animale were still gaining weight. However, the respiratory quotient did not alter significantly during the course of the dystrophy until severe muscle degeneration was observed; then it was lowered. The lowering of respiratory quotient was probably due to inanition in the terminal etages of dystrophy. Glycolysis was found to be higher by 50 per cent (120). Weinstock (249) announced that particulate matter of liver homogenates from tocopherol deficient rabbits respired at increased ratee. These findings indicated that vitamin E-deficiency fundamentally affected the oxidative metaboliem of muscle. Although no gross or microscopic lesions could be detected in the liver, still the liver metabolic function wae altered. These facts were confirmed by Rosenkrantz (207), who found that the oxygen coneumption of various tiesuee ae skeletal muscle, heart muscle, kidney, liver, and adrenal gland was found to be significantly higher. The tiseues were obtained from vitamin E-deficient rabbits by sacrificing them when the urinary creatine levels were below 20 milligrams in 24 hours. The higher oxygen consumption of adrenal gland was reported to be due to vitamin E deprivation in the tiseuee, strese complication, or a combination of both factors. Alteration in the adrenal respiration was observed in advance of all pathological activity. Contrary to what hae been found in muscles, addition of alpha-tocopherol to liver elices from deficient rats, either ae an emuleion or in water eoluble form, was reported by Schwarz (212) to have no effect in reducing oxygen consumption. However, the

injection of an emulsion of dl-alpha-tocopherol via portal vein procured an immediate disappearance of the symptoms (201, 202).

Telford (237) reported that there was no increase in respiration rate of the thyroid gland in vitamin E-deficiency.

Alterations in Enzyme Activity

The earliest report of increased succinoxidase activity for dystrophic muscle was by Houchin and Mattil (115). A decrease in the activity of the ketoglutaric dehydrogenase and isocitric dehydrogenase was demonstrated by Rosenkrantz (207) and Bassy and Rosenkrantz (9). Working with the leg muscles of vitamin E-deficient rabbits, Allen (3) found an increase in cytochrome oxidase and reductases, particularly in the terminal stages of deficiency. That the diphosphopyridine nucleotide-cytochrome-c reductase activity of the liver, skeletal muscle, and heart muscle was lowered in vitamin E-deficiency in chicks, rabbits, and rats was shown by Nason and Lehman (176). Phosphoglucomutase activity was decreased considerably in white muscles of vitamin E-deficient chicks, whereas the red muscles remained unaffected. The decline in phosphoglucomutase activity paralleled the severity of muscle lesions. Phosphorylase activity also decreased in white muscles (100). It was concluded that enzymatic activity concerned in glycolysis, Krebs' cycle and electron transport decreased in vitamin E-deficient animals.

Adenosine triphosphatase activity of muscle homogenates showed a slight trend towards lower values in vitamin E-deficiency in guinea pigs (220).

Blincoe and Dye (18) recorded higher levels of serum glutamic oxolacetic transaminase (SGOT) in white muscle disease of calves and lambs as compared to normal controls. A comparison of SGOT levels of naturally occurring cases of white muscle disease with that of cod liver oil induced muscular dystrophy in calves and lambs was made by Kutler and Marble (134). The SGOT values in artificially induced muscular dystrophy were considerably higher. Dystrophic signs appeared in calves when the SGOT rose to 295 to 890 Sigma Frankel units per ml. and in lambs when 2,000 to 3,000 units per ml. was reached. The levels of SGOT rose in lambs even before clinical symptoms of dystrophy were apparent. A direct relationship was shown by Swingle et al. (235) between the amount of transaminase in serum and ensuing incidence of muscular dystrophy in lambs of seven to fourteen days of age. Blincoe and Marble (18) showed a linear correlationship between SGOT values and lactic dehydrogenase in serum. Alkaline phosphatase increased only in the terminal stages. Walker (248) studied the SGOT levels in a flock of lambs affected with white muscle disease and found higher levels.

Studying the addition of dieting selenium to lambs in an endemic area as a preventive measure for white muscle disease, Lagace et al. (136) reported that the SGOT levels rose to a peak by six weeks of age and then declined by the tenth week. Similar attempts with administration of vitamin E to lambs revealed that the initial elevation of SGOT levels was less (136). Evaluating the effect of oral or parenteral administration of selenium in white muscle disease of lambs, Lagace (135) used

SGOT values as an index, which decreased with treatment.

Changes in Electrolyte Concentration of Tissues, Blood and Urine

Fenn and Goettsch (73) were the earliest to report increased sodium, and decreased potassium and magnesium content of the residual muscle in muscular dystrophy of rabbits. Zuckerman and Marquardt (264) conclusively showed that muscle potassium, magnesium, and adenosine triphosphate levels decreased while sodium, calcium, and chloride content increased significantly in dystrophic rabbit muscles. Young et al. (262) reported that dystrophic skeletal muscle of the rat contained 12 to 23 per cent less potassium and 68 to 70 per cent more sodium, and that cardiac muscle differed little in its potassium content. Baldev (7) recorded that lamb cardiac muscle with white lesions of white muscle disease contained more magnesium than those of other affected muscles or those not affected.

No significant differences were reported in the electrolyte content of erythrocytes and serum by Zuckerman and Marquardt (264) in dystrophic rabbits. However, Baldev (7) reported a depression in serum magnesium levels in white muscle disease of lambs, although no alterations were noticed in other electrolytes. Maplesden and Loosli (147) demonstrated that the serum magnesium levels were lowered in dystrophic calves raised on a diet containing cod liver oil. Dehorty et al. (47) investigated the effect of adding cod liver oil, alpha-tocopherol, and magnesium, singly or in combination, to whole milk diets of calves and

reported a decrease in serum magnesium levels when alpha-tocopherol and cod liver oil were added to the diet. Earlier Blaxter and Wood (16) reported decreased serum magnesium levels in calves made dystrophic by the addition of cod liver oil, and attributed the calcification of soft tissues to magnesium deficiency resulting from avitaminosis E.

Changes in Cellular Constituents of Blood

Very few reports describe any changes in cellular constituents of blood in avitaminosis E. Maplesden and Loosli (147) reported that the hemoglobin concentration and total leucocyte count did not vary much in vitamin E-deficient calves as compared to the vitamin E supplemented group. However, their data showed that there was a slight elevation in granulocytes and a decrease in lymphocytes in the E-deficient animals. Borgman (21) recorded no variations in erythrocyte, total leucocyte, and hemoglobin concentration of the E-deficient calves as compared to the controls.

Cholesterol Metabolism in Vitamin E-Deficiency

Vitamin E-deficient diet increased serum and muscle cholesterol levels (16, 44, 48, 167, 168, 228). Smith *et al.* (228) and Shull *et al.* (219) supplemented vitamin E-deficient diets with antioxidants and found that although the onset of dystrophic symptoms was delayed, the plasma and muscle cholesterol level elevations were not altered. Supplementing a deficient diet, however, did decrease the serum cholesterol levels. Deuel *et al.*

(48) stated that plasma and muscle cholesterol and total muscle lipids levels were elevated in rabbits raised on vitamin E-deficient synthetic diets. Free and esterified cholesterol levels were increased in plasma, whereas free cholesterol only was increased in the muscles (48). No changes were reported in the total lipid or cholesterol content of the liver. Increased esterified cholesterol fraction was seen mainly in plasma of rats and guinea pigs (48).

Shull et al. (219) demonstrated that the rise in cholesterol concentration of plasma precedes the onset of muscular dystrophy in guinea pigs.

Tissue Ubiquinone Levels in Avitaminosis E

The ubiquinone concentration in tissues from vitamin E-deficient animals has been a subject of controversy. Moore (162) and Morton and Phillips (170) found no indication of a relationship between the vitamin E status of the rat and the ubiquinone levels of its tissues. These results have been contradicted by Green et al. (91) who demonstrated an increase in ubiquinone levels in various tissues of the rat, rabbit, and avian species. Edwin et al. (66) and Diplock et al. (61), in their work with rats, conclusively showed that the ubiquinone content decreased when rats were maintained on a vitamin E-deficient regimen. They found higher ubichromenal concentrations. Administration of selenium significantly elevated ubiquinone concentration in all tissues except uterine (61). Selenium was shown by Edwin et al. (66) to increase ubiquinone in the same

way as alpha tocopherol. That their actions are complimentary to each other was demonstrated by Diplock et al. (61, 62). Ubiquinone content of heart, breast, and thigh muscles was elevated in poults following selenium administration (62). He found that muscles of poults contain greater ubiquinone amounts as compared to mature turkeys on a stock diet. Phillips (189) observed that the ubiquinone levels of tissues were related to the vitamin E status of the animal. Recent studies of Lee et al. (141) contradicted the findings of Green et al. (81), Diplock et al. (61), Edwin et al. (66), and Phillips (189); Lee's studies failed to provide any evidence for the dependence of ubiquinone levels on vitamin E status of rats, rabbits, or for any functional interrelationship between these compounds. A non-tocopherol reducing substance, believed to be ubichromenol 45, was found in greater concentration in vitamin E-deficient animals by Crider et al. (42) and Edwin et al. (66). Although ubichromenol had no influence on muscular dystrophy of vitamin E-deficient rabbits (65), still Johnson et al. (128) showed that administration of ten milligrams of ubichromenol subcutaneously in rats for five to seven days after mating successfully prevented resorption of fetus and promoted a higher rate of live young at birth.

Sandergaard et al (210) reported that although injection of ubiquinone 50 slightly delayed the onset of encephalomalacia in chicken, the incidence or severity of muscular dystrophy was not affected. However, ubichromenol reduced the incidence of encephalomalacia and muscular dystrophy by about one-third. The

severity of the muscular dystrophy was likewise reduced. Whether ubichromenol has a primary metabolic effect or merely spares vitamin E reserves remains unknown. Smith (225) revealed that intravenous administration of hexahydrocoenzyme Q_4 at 20 milligrams per kilogram body weight per day for five successive days was effective in preventing muscle weakness and creatinuria. The quinone of tocopherol had no effect. Hexahydrocoenzyme Q_4 is claimed to be superior to alpha-tocopherol (225).

Relationship of Vitamin E to Endocrine Glands

Deprivation of vitamin E from diets of animals resulted in structural changes in adrenal cortex (108, 23).

Intravenous injection of adrenocorticotropic hormone before and after daily oral treatment with 600 milligrams of alpha-tocopheryl acetate for six days did not affect the 17-hydroxy corticoids in plasma or the 17-ketosteroids in urine (33). Vitamin E-deficient animals showed histological changes similar to those given high doses of vitamin E. Hence, Heinsen (105) concluded that the response may be due to a general adaptation syndrome.

Experimental evidence provided by Borgman and Underbjerg (22) showed that when adrenal cortical hormones were injected daily into vitamin E-deprived rabbits, muscular dystrophy appeared earlier with more severity than in those not given the hormone. Fifty per cent of such rabbits showed regressive changes in the zona glomerulosa, but only one had hypertrophy. Stress applied in the form of low atmospheric pressures, either

acutely or chronically, lowered blood vitamin E levels, total lipids, and total cholesterol in the rat (191). Daily subcutaneous injection of 220 milligrams of alpha-tocopherol per 100 gram body weight for six days produced no change in the weight of adrenal glands or in their ascorbic acid levels. The same dosage level of alpha-tocopheryl-quinone decreased adrenal weight and increased the ascorbic acid level (188). Raymondi (203) observed that daily parenteral administration of 75 milligrams of vitamin E per kilogram body weight to rabbits for 40 days elicited a clear influence of the vitamin on the glomerular and fascicular zones with cortical changes related to hyperemis. In this respect the action of vitamin E was comparable to the adrenocorticotropic hormone. Adrenal weights were increased by the high doses of vitamin E (231). When large doses of vitamin E were administered to young male rats, the weight of adrenals increased while the weight of the thymus decreased (78).

Ellis (68) reported that alpha-tocopherol did not prevent the development of voluntary muscle lesions in rabbits given cortisone daily for 7 to 21 days. The lesions were similar to those described in rabbits fed a potassium deficient diet (68).

Rosenkrantz (207) studied the oxygen uptake of the adrenal cortex and reported an increased oxygen uptake in vitamin E-deficient rabbits even before signs of muscular dystrophy appeared. Special staining techniques indicated an increase in adrenocorticosteroid production.

Administration of vitamin E increased the elimination of 17-ketosteroids (105, 226).

Hillman et al. (109) raported that treatment with adrenocorticotropic hormone tended to raisa the tocopherol lavels. Rats maintained on a vitamin deficient regimen produced an increase in tha numbar, aize, and aecratory activity of the peripheral gonadotrophs, which are believed to ba the cells secreting follicular stimulating hormone. The central gonadotrophs, which are balieved to produce the leutinizing hormone, wara slightly stimulated (93). Ingelman and Sundberg (121) raported that the gonadotropic content of the anterior pituitary lobe decreaaed significantly in vitamin E-deficient animals thus suggasting a primary hypophyaeal disturbance. Okumara (179) noticed no appreciable change in the pituitaries of mice after administration of vitamin E for one or two daya. However, Suardi (231) reported that as the vitamin E doae waa increaaed the pituitaries showed higher numbera of basophilic cells and fewer chromophobic cells. After bilataral adrenalectomy the basophilic cells increased in number (230). Male and femala mice showad aarlier differentiation of tiaaues when injectad with one milligram of vitamin E per gram body weight evary other day until ten days of aga. Howaver, aftar 60 to 80 days of age, pituitarias of treated animals were similar to thoae of controla. Bata cells exhibited early differentiation (238).

Verzar (245) commented that the antarior pituitary posseasad the highast concentration of tocopherol in the body, and was of the opinion that the lack of vitamin E would laad first to a dacreased production of tha anterior pituitary hormonas.

However, the findings of Diplock et al. (61) revealed that, contrary to expectations, the pituitary glands contained little tocopherol and ubiquinone after the administration of vitamin E to tocopherol deficient rats.

Normal levels of thyrotropin in the anterior pituitary were reported by Bomskov and Schneider (20). Data on the weights of thyroid glands of both sexes in avitaminosis E appeared to differ. Barrie (8) reported that the thyroid glands of both sexes of rats were hypoplastic. Differing from this observation, Biddulph and Meyer (10) recorded that the thyroid glands of vitamin E-deficient male rats were increased in weight while the females were unaffected. Observations of Telford et al. (237), however, indicated no histological abnormalities of the gland in deficient suckling rats or in chronically deficient adult mice. The parenchyma of the thyroid exhibited signs of hypofunction when small or no doses of tocopherol were administered for several days (230).

Compared to normal values, hyperthyroid humans have a lower plasma tocopherol level while hypothyroid persons a higher level (41). Vitamin E administered at excessive levels reduced the creatinuria and oxygen consumption in rats under thyroxine therapy (25). Symptoms of avitaminosis E were aggravated by the administration of thyroprotein to chickens (99). Telford et al. (237) reported that the rate of respiration was not affected in vitamin E-deficiency.

The pancreas in animals receiving vitamin E showed slight insular hyperplasia (229). Vitamin E acted synergistically with

insulin in increasing both glucose uptake and glycogen synthesis, but not with lactic acid formation (87).

Injection of 50 milligrams of vitamin E daily for ten days eignificantly increased production of glucagon; smaller doses produced no change. The ratio of alpha cells to beta cells in the pancreas increased about 30 per cent, and the alpha cells appeared hyperplaetic with a ewelling of cytoplasm. Nuclei of alpha cells contained two or three nuclei with chromatin in a grose reticular arrangement (123). Kokol and Chelmin (133) reported that vitamin E apparently retarded the insulin eystem of the pancreas as was evidenced by decreaeed sugar tolerance in both normal and alloxan diabetic rabbits.

Adminietration of 200 milligrams of alpha-tocopherol daily for four days increased the urinary estrogen output of females by 43 per cent. Estrogens were not found in the urine of diestric guinea pigs in either vitamin E-deprived or control groups (121).

Interrelationehip Between Selenium and Vitamin E

A third factor, besidee vitamin E and cystine, which was involved in the prevention of dietary liver necrosis in rats, wae named Factor 3 by Schwarz (212). This factor prevented vitamin E-deficiency in chicks raieed on Torula yeast which causee growth failure, exudative diathesis, and death (218). Selenium was identified as an integral constituent of Factor 3 (215). Selenite, selenocyetathionine, and elemental selenium were also shown to be equally effective in curing or preventing

dietary liver necrosis in rats and exudative diathesis in chicks (216).

While reviewing Factor 3, selenium, and vitamin E, Schwarz (213) distinguished three categories of disease: (1) those caused purely by a vitamin E-deficiency; (2) those caused purely by Factor 3 or selenium; and (3) those caused by a simultaneous lack of both vitamin E and selenium. The lack of Factor 3 or of vitamin E often produced a relatively mild, chronic type of disease, while a simultaneous deficiency of both lead to acute impressive tissue damage leading to death. White muscle disease of calves and lambs falls in the last group, and selenium was reported to be as effective as vitamin E against this condition (135, 173). Oldfield et al. (163) stated that selenium appears to be essential for growth. The changes in tissue mineral content in muscular dystrophy suggest that the syndrome is not a simple vitamin E-deficiency. Contrary to what happened in calves and lambs, Factor 3 active selenium compounds did not prevent or alleviate muscular dystrophy of rabbits caused by tocopherol deficiency (63). An effort to differentiate the action of selenium and alpha-tocopherol as nutrienta preventing muscular dystrophy was made by Maplesden and Loosli (147). Interestingly enough, they reported that addition of one part per million of selenium to the diet containing alpha-tocopherol and cod liver oil did not prevent the development of muscular dystrophy, and that alpha-tocopherol and cod liver oil fed calves developed degenerative changes in skeletal muscles and Purkinje fibers of the heart. Schwarz (213) remarked that

neither selenium nor vitamin E could substitute for one another. So it seems improbable that selenium has any sparing action for tocopherol. In this connection Bieri et al. (13) reported that the rate of depletion of vitamin E from tissues was not influenced by selenium. Contrary to the previous findings, Proctor et al. (197) reported that neither vitamin E in excess of supposed requirements nor selenium prevented muscular dystrophy in rabbits fed diets containing Torula yeast, cerelose, lard, vitamin and salt mixtures, 20 per cent linseed oil, wheat bran, or 40 per cent cooked beans.

The effect of selenium on tissue constituents of vitamin E-deficient animals was investigated (60, 66). Administering selenium to vitamin E-deficient rats significantly enhanced ubiquinone concentration, and in this respect, vitamin E and selenium were complementary to each other (60, 66). There also appeared to be a biosynthetic relationship between tocopherol, selenium, ubiquinone, and ubichromenol (66).

Selenium fed to chicks at 0.05 or 0.1 parts per million as sodium selenite successfully replaced vitamin E or dried brewer's yeast in preventing an increase in red cell volume and a reduction in hemoglobin content, erythrocyte count, total serum proteins, and albumin globulin ratio (199). The addition of selenium failed to improve hatchability or chick mortality, but the substitution of brewer's yeast for Torula yeast prevented the high incidence of early chick mortality (124). The work of Calvert et al. (32) showed that in chicks neither selenium factors nor alpha-tocopherol supplementation in dystrophic

producing diets completely prevented muscular dystrophy.

Avitaminosis A

The lesions and changes observed in vitamin A-deficiency are so numerous and diverse that they are difficult to group or provide a clear and unified picture. The final pathological picture will usually be influenced by animal species, duration of deficiency, sex, and stresses to which the animal is subjected. Bacterial environment may alter the final picture, since in most cases death supervenes as a result of secondary bacterial infection even before the full manifestation of the deficiency state. Pure avitaminosis A can only be experimentally produced. However, as with most other vitamins, a deficiency state may sometimes result, not from the lack of vitamin A in the diet, but from defective absorption and metabolism or increased excretion. Disease conditions with fevers may also exert striking effects on the metabolism of vitamin A.

Wolbach and Bessey (254) stated that the specific changes would be found in the epithelia whose cells have a secretory function in addition to the role of a covering layer whose functional cells are without power to divide. These include the epithelia of the digestive, respiratory, genito-urinary, special senses such as eyes, and miscellaneous systems like the thyroid.

The relationship between levels of vitamin A and the changes noticed in deficient tissues has not been established as yet. Although the important function of vitamin A in the mammalian

organism is to maintain the integrity of epithelial structures, the biochemical mechanism by which it achieves this end is as yet obscure. A great deal of knowledge has accumulated regarding the relationship of vitamin A to the functional activity of rod and cone cells of retina and the visual processes.

According to Radhakrishnarao (198), rabbits placed on a vitamin A-deficient diet had sore eyes, clouded cornea, and xerophthalmia of varying degrees involving cornea or conjunctiva. In addition, the deficient animals exhibited demyelination of peripheral nerves, particularly sciatic, femoral, and brachial plexus. However, anatomical changes in nerves were not as severe as those in the eye. The affected nerve fibers demonstrated annular degeneration with dark rings of degenerated myelin surrounding the axis cylinder. In more advanced stages of nerve degeneration, fine or coarse granules of disintegrated myelin were found.

Young rabbits born to deficient mothers were reported by Millen et al. (158) to have hydrocephalus with distension of lateral ventricles. Owing to fluid pressure in the ventricles, the cortex and white matter of brain substance were reduced to an extremely thin layer lining the interior of the skull. Herniation of the brain tissue through the skull and of the cerebellum through the foramen magnum were noticed in some cases. They thought that hydrocephalus was produced in young animals because of stenosis of the aqueduct, although excessive production of cerebrospinal fluid pressure might be more important.

In studying the effects of rations producing blindness in

bovines, Phillips and Bockstedt (190) reported that when the rations were fed to rabbits, stenosis of optic foramen was not observed, probably due to the anatomical arrangement of the skull. However, blindness and ataxia developed in rabbits on vitamin A-deficient rations. Involvement of locomotion, equilibrium, and sight were common. The first symptoms appeared in the eye as a fleeting or persistent ophthalmia, then keratitis progressing from Bitot's spots to opacity and blindness. Keratitis was an interstitial type which gave a ground glass appearance. In most cases light reflex was sluggish. The condition was accompanied by progressive ataxia and loss of equilibrium. Partial paralysis of the legs, most frequently in front quarters, was noticed in some instances, and normal position of the head was lost. In later stages, diarrhea and salivation were common. Growth was retarded in severe cases, and the animals lost weight.

Histopathological studies of tissues indicated a mild parenchymatous degeneration of proximal convoluted tubules with focal and interstitial proliferation in kidneys. Reaction of Kupffer's cells, congestion in liver, and involvement of spleen in a few cases were described. Myelin degeneration of sciatic nerve and brachial plexus, and atrophy of Purkinje cells of cerebellum were the changes observed in the nervous system. Lacrimal glands were inflamed, and molar teeth were irregularly worn out.

Lamming (137), who experimentally produced avitaminosis in rabbits by feeding a vitamin A-deficient diet, reported that the first sign of disorder was the retraction of the head in most of

the animals and paralysis of the hind quarters in some. Although these symptoms were common, emaciation without head retraction was noticed in a few instances. Hydrocephalus also developed in some with considerable stenosis of the aqueduct. Herniation of cerebellum was observed, although there was no deformity of foramen magnum or the back of the skull bones. The optic nerve showed distinct constriction in the region of the optic foramen. Cerebrospinal fluid pressure which increased might have caused the stenosis.

In another experiment Lamming et al. (139, 140) studied the effects of avitaminosis on reproductive phenomena in rabbits and reported that incipient vitamin A-deficiency in rabbits produced premature degeneration of ova and a reduced number of fertilized ova when examined at 40 hours and at four days post coitus. The number of uncleaved eggs was increased. They reported that in incipient vitamin A-deficiency the percentage of rabbits that mated early and the number of conceptions were decreased. But, the number of corpora-lutes did not decrease in pregnant animals. In vitamin A-deficiency the loss of ova before implantation reduced the litter size significantly. These reports indicate that vitamin A may be required during intrauterine and extrauterine life. The weights of fetuses were lower.

Vitamin A-deficiency was shown by Lowe et al. (142) to increase the concentration of liver ubiquinones in rats. However, Morton and Phillips (170) could not confirm this in guinea pigs. Heaton et al. (101) and Moore and Sharman (166) confirmed the observations of Lowe et al. (142). Investigations of Gloor and

Wiss (84) and Phillipe (189) indicated that the incorporation of labelled mevalonic acid into ubiquinone was greatly enhanced by vitamin A-deficiency. Whether the increase in liver ubiquinone was only a symptom of fetty liver or whether it was due to enhanced ubiquinone synthesis was questioned. It was shown that increased ubiquinone in liver was a result of metabolic alteration and not a secondary effect resulting from tissue damage. On further examination, Lowe et al. (142) found that neither in the chick nor in the guinea pigs were the ubiquinones elevated in the liver.

Both increased (200) and decreased (81) levels of blood cholesterol have been reported in vitamin A-deficiency. Wood (259) recently presented evidence that vitamin A, when fed to chicks in large amounts with cholesterol, accounts for the major part of the hypocholesterolemic effect of lingcod-liver oil. However, the work of Weitzel et al. (251) indicated little change in blood cholesterol levels in aged atherosclerotic hens fed large doses of vitamin A, although a marked regression of atheromatosis was noticed. Phillips (189) interpreted his data on vitamin A-deficient rats by saying that a block in the later stages of cholesterol biosynthesis caused a feed-back response increasing the synthesis of ubiquinone as well as squalene. The effect of vitamin A-deficiency on cholesterol, squalene and ubiquinone synthesis in guinea pigs appears opposed to that observed in rats. Gloor and Wiss (84) suggested that an interaction between vitamin A and ubiquinone takes place. However, the experiments of Phillips (189) strongly indicate that the increased

ubiquinone found in rat liver was due to suppression of cholesterol synthesis; thus, there was accumulation of squalene which, in turn, increased the level of farnesyl pyrosphosphate forcing the reaction towards ubiquinone formation.

Sure et al. (233) reported that in the early stages of deficiency rats had lowered hemoglobin values or erythrocyte counts, and when xerophthalmia developed, the hemoglobin level and erythrocyte count increased. Observations on blood constituents by Frank (80) indicated that there was a lowered hemoglobin level and erythrocyte and leucocyte counts with a higher neutrophiles to lymphocytes ratio. In two human infants having xerophthalmia, the total leucocyte count and the neutrophiles to lymphocyte ratio was lowered (80). In the early stage of vitamin A-deficiency in rats, Crim and Short (43) observed that the Arneth index showed a shift to the right. From their observations on experiments with rats, Abbot and Ahman (1) concluded that a prolonged deficiency produced a decrease in the neutrophile count, increase in the number of juvenile cells, and an increase in the ratio of large to small lymphocytes. The first sign of deficiency, however, was a marked rise in neutrophiles and an equally steep fall in lymphocytes.

Frank (80) found that in both rats and human infants vitamin A-deficiency interfered with blood clotting mechanism; fibrinogen content and coagulation time were increased.

Moore (164), who thought that the calcium metabolism might be affected in vitamin A-deficiency, found contrary to their expectations that the serum calcium content was normal in calves

in avitaminosis A.

Relationship Between Vitamin A and Endocrine Secretions

The earliest report that adrenal cortex was concerned with the mobilization of vitamin A was that of Chevallier et al. (34). Later his finding was confirmed by Clark and Colburn (36) who stated that injections of cortisone reduced the liver reserves of vitamin A by 20 per cent.

Popper and Greenberg (192) showed that the adrenal cortex contained a high concentration of vitamin A in the zona fasciculata, which was thought to be the source of glucocorticoid hormones. A suggestion was made by Erstoff (70) that the deficiency of adrenal cortical hormones was possibly caused by vitamin A-deficiency.

Johnson and Wolf (127) demonstrated that weanling rats adrenalectomized and receiving vitamin A but no cortisone grew normally and exhibited no symptoms of vitamin A-deficiency. Glycogen synthesis was, however, depressed; whereas the animals receiving cortisone without vitamin A came down with all symptoms of vitamin A-deficiency and produced glucose normally. The results mentioned above indicated that vitamin A-deficiency led to a change in the adrenal gland so that it was unable to produce glucogenic hormone. In another experiment Wolf et al. (256) stated that incorporation of acetate into liver glycogen was lowered by vitamin A-deficiency as much as by adrenalectomy in spite of simultaneous treatment with adrenocorticotropic

hormone. These results confirmed the reports of Lowe et al. (142) that phospholipid appeared in the zona glomerulosa and disappeared in zona fasciculata in vitamin A-deficient animals. This process became more pronounced as the deficiency progressed, until in severe deficiency zona fasciculata contained no phospholipid, whereas zona glomerulosa stained heavily for it.

In the rat and rabbit, corticosterone is the predominant steroid, whereas in man, monkey, cat, and dog, cortiaol predominates (104). When adrenal alices from vitamin A-deficient and normal animals were incubated with adrenocorticotropic hormone, niacin-adenine-dinucleotide phosphate (NADP), and glucose, corticosterone secretion of the vitamin A-deficient animal was about half of that of the normal animals (241). The effect of addition of vitamin A to the medium was not studied. However, curative experiments with vitamin A administered to the animal prior to sacrificing demonstrated that the ability of the adrenal gland to secrete corticosterone was restored essentially to normal (114).

Lamming and Salisbury (138) indicated the possibility that vitamin A might be concerned with the synthesis of steroid hormones, particularly progesterone. Later Grangaud and Conquy (89) reported a role of vitamin A in progesterone biosynthesis from pragnanelone. In severely deficient animals progesterone biosynthesis was depressed, but in more mildly deficient animals there was no block in progesterone biosynthesis nor in the conversion of pregnanelone to progesterone, even though there was still a depression of corticosterone synthesis (127).

Cystic pituitary glands were observed by Madsen et al. (150) in young cattle which were either suffering from vitamin A-deficiency or had a history of severe depletion in life. The cysts were present in the residual lumen or in the posterior lobe and caused compression of the gland and injury to the glandular parenchyma.

Wolf et al. (256) demonstrated that the pituitaries of vitamin A-deficient rats retained their gonadotropic power. That the gonadotropic activity of the pituitary was increased in castration was shown by Sutton and Brief (234). This increase which was greater in males than in females was regarded as a compensatory reaction to the primary injury to the gonads.

The relationship of thyroid to vitamin A has been extensively studied. The effect of thyroxine in inducing vitamin A-deficiency, particularly at the onset of xerophthalmia was shown by Sure and Buchanan (232), and on vaginal keratinization by Greaves and Schmidt (90). In their experiments with live rats, Wiese et al. (252) demonstrated that the lives of vitamin A-deprived rats were prolonged by the administration of thiouracil. Cooper et al. (39) reported that thiouracil could improve the growth rate of chickens given small doses of vitamin A. That thyroxine aggravated the effects of avitaminosis A (155) agreed well with the fact that oxygen consumption of rats increased in vitamin A-deficiency, as reported by Blaizot and Benac (14). It was reported by Sheet and Struck (218) that massive doses of vitamin A concentrates reduced the metabolic rate in rats given thyroxine. An antagonism between thyroxine and vitamin A seems

to be existent.

It was indicated by Fesold and Hiedemann (72) that thyroxine was concerned in the conversion of carotene to vitamin A. In the blood of human patients with hyperthyroidism the carotene and vitamin A levels were low (250). Drill and Truant (64) opined that carotene cannot be converted to vitamin A by thyroidectomized rats, and their results were confirmed by Johnson and Bauman (126). Arnrich and Morgan (5) showed that thiouracil had no effect on vitamin A stores, at least when high doses of carotene were given. Bieri and Schultze (12), however, were unable to detect any significant difference in the storage of vitamin A by normal rats and by others treated with thiouracil.

Thyroxine may increase the storage of vitamin A, whether derived from carotene or from preformed vitamin (160). However, that the expenditure of vitamin A seemed to be increased by thyroxine during restriction to a deficient diet was demonstrated by Johnson and Bauman (126).

Interrelationship Between Vitamin A and E

From the standpoint of nutrition the intimate relationship of vitamin E with vitamin A was first reported by Moore (165), who noticed that vitamin A reserves of tocopherol deficient rats were invariably lower by two to tenfold than those of the controls given vitamin E. That the effect was partly due to a protective action of vitamin E on the vitamin A reserves was shown by Davies and Moore (46). In their experiments with chicks Dam *et al.* (44) stated that the vitamin reserves were depleted

progressively when they were kept on a vitamin E-deficient diet. Most of the later work has shown the interrelationship of vitamins A and E in storage, utilization of each other, and on abortions. That small amounts of tocopherols administered to rats with marginal amounts of vitamin A increased the growth promoting action of vitamin A and lengthened their survival was revealed by the interesting studies of Hickman et al. (107). The mechanism by which the effect was brought about was explained to be due to inhibition of oxidation of vitamin A. Absorption of vitamin A from the intestines was not increased when vitamin E was given orally or parenterally (176). When a single dose of vitamin A was given orally to rats deficient in vitamins A and E, the absorption was less. When a similar dose was administered to rats having adequate stores of vitamin E, relatively more vitamin A was absorbed (66). Dicks et al. (49) stated that in calves deficient in both vitamins A and E, either very low or very high levels of vitamin A given orally could be effectively absorbed, as compared to intermediate doses.

Comparatively little work has been done on the effect of vitamin A on the absorption of vitamin E. Tocopherol levels in various tissues were reported to be depressed when increased vitamin A was administered to rats (66). Similar results were reported in cattle by Dicks et al. (49).

Symptoms of experimental hypervitaminosis A were reported to be depressed by giving supplementary doses of tocopherol (41). However, diphenyl-p-phenylene-diamine (DPPD) supplementation accelerated the progress of the condition. This showed that the

protective action was not due to its antioxidant effect. Chevrel and Cornier (34) reported that external signs of vitamin A-deficiency in the rabbit appeared only when the animals were deficient in vitamin E. A remarkable synergism between vitamins A and E was noticed in reducing creatinuria in rats. In vitamin E-deficient rats the dose of vitamin E required to reduce creatinuria to zero was less when vitamin A was administered simultaneously than when alpha-tocopherol was given alone (30). In severe vitamin E-deficiency neither of the vitamins alone was efficient in reducing creatinuria. Edwin et al. (66) reported that rats deficient in vitamins A and E tended to survive longer than rats deficient only in vitamin A.

Moore (161) reported earlier that vitamin E had less effect on the storage of vitamin A derived from carotene than on the storage of preformed vitamin A. When vitamin A-deficient rats were administered carotene, increased quantities of tocopherol administration led to diminished storage of vitamin A in the liver (126). Depending on the dosage of supplementary tocopherol the storage of vitamin A varied. Hebert and Morgan (102) suggested that tocopherol influenced the actual absorption of carotene. Bieri (9), who studied the utilization of a single dose of carotene by rats deficient in vitamins A and E, reported that tocopherol increased neither growth nor vitamin A storage. His experiments suggest that an absorption rather than a protective effect may account for the interrelationship between these two vitamins.

MATERIALS AND METHODS

Sixteen New Zealand white rabbits, five to six weeks of age and averaging 1,098 grams, were selected and randomized into four groups on the basis of sex and weight. All the animals were maintained on a semi-purified diet. One group was raised on a complete diet containing all the necessary ingredients, the composition of which is given below, and served as controls; second group on a diet deficient in vitamin A; third group on a diet deficient in vitamin E; and fourth group on a diet deficient in the vitamins A and E. The groups deprived of one or both of the vitamins were maintained on the diet until visible symptoms of deficiency were noticed and supported by other criteria.

Semi-purified Diet

A modification of the diet used by Borgman (21) was used and contained the following ingredients:

Ingredients	Per cent composition
Vitamin free casein	30.00
Alphacel (non-nutritive bulk)	15.00
Molecularly distilled lard	20.00
Corn starch	6.70
Sucrose	9.25
Dextrose	8.00
Briggs' salt mixture (described below)	6.00
Potassium acetate	2.50
Magnesium oxide	0.50
Linoleic acid	0.30
Linolenic acid	0.30
Choline chloride	0.30
Inositol	0.40
Ascorbic acid	0.50
p-Aminobenzoic acid	0.25

Ingredients	Milligrams per 100 grams of diet
Alpha-tocopherol acetate	10.00
Vitamin "A" acetate	0.60
Vitamin D_3	0.004
2-methyl-1,4-naphthoquinone	0.20
Thiamine hydrochloride	1.60
Pyridoxine hydrochloride	1.60
Riboflavin	1.60
Nicotinic acid	20.00
Calcium pantothenate	4.00
Folic acid	0.60
Biotin	0.60
Vitamin B_{12}	0.004

Composition of Briggs' Salt Mixture (26)

Ingredients	Per cent composition
Calcium carbonate	25.00
Dipotassium hydrogen phosphate	15.00
Disodium hydrogen phosphate	12.17
Tricalcium phosphate	23.33
Sodium chloride	14.67
Magnesium sulfate - hydrated - 7 H_2O	8.33
Manganese sulfate - hydrated - 1 H_2O	0.53
Potassium iodide	0.066
Zinc carbonate	0.033
Cupric sulfate	0.033
Ferrous sulfate	0.838

Collection and Preservation of Materials for Analysis

The rabbits were put in individual metabolism cages one day a week, and their food consumption, water intake, and volume of urine were measured. Body weights of the rabbits were recorded once every week on the same day the rabbits were taken from the metabolism cages. The urine samples of each group were pooled and a representative sample of about 200 milliliters was preserved in an amber colored bottle with eight drops of chloroform

and frozen until analyzed. At 15-day intervals 5 ml. of blood from each rabbit were collected by cardiac puncture, and the pooled serum from each group was frozen and stored until analyzed. Weekly blood samples were collected from ear vein for rbc determinations. After sacrificing the animals at the end of the trial period, skeletal muscle, liver, heart, and adrenal glands were collected for tissue respiration studies and ubiquinone estimation. Immediately after sacrificing, a section of skeletal and heart muscle was taken for respiration studies, and the other tissue slices were placed in polyethylene bags and frozen in a freezer for later investigational use.

Biochemical Techniques

Urinary Creatine and Creatinine (Fister, 76). The urine was filtered and diluted twenty times with distilled water. A 2 ml. portion of the diluted urine was taken in a 50 ml. test tube containing 16 ml. of N/12 sulfuric acid. The contents of the tube were mixed by gentle rotation, and 2 ml. of a 10 per cent sodium tungstate solution was added slowly with mixing. Five ml. of the mixture were pipetted out into 2 graduated centrifuge tubes. One tube was used for determination of total creatinine and the other for preformed creatinine.

For determination of total creatine the graduated centrifuge tubes were covered with tinfoil and kept at 155° Centigrade in a hot air oven for 10 minutes, then cooled under tap water and the volume was adjusted to 5 ml. with distilled water. A blank with 5 ml. of distilled water was employed. Two and

one-half ml. of a freshly prepared alkaline picrate reagent were added to each of the tubes for preformed creatinine and total creatinine determination and blank. The alkaline picrate reagent was prepared by mixing 5 ml. of one per cent picric acid with 2.5 ml. of 2.5 N. sodium hydroxide and made up to 25 ml. with distilled water.

A standard curve was plotted with optical densities given by known quantities of creatinine treated in a similar manner as the unknown.

The Coleman Model 14 Spectrophotometer[1] was set at 100 per cent transmittancy at 520 millimicrons wavelength, with the distilled water blank. The optical densities were read for each of the solutions including the blank. The optical density of the blank was deducted from the readings of the unknown, and the concentrations of creatinine were read from the standard curve already prepared and the results expressed in mg. per cent. The urinary creatine was obtained by subtracting the preformed creatinine from the total creatinine.

A ratio was computed by dividing the creatinine concentration into the creatine concentration and multiplying by hundred:

$$\frac{\text{creatine} \times 100}{\text{creatinine}} = \text{creatine:creatinine ratio.}$$

Sodium and Potassium in Urine. A Coleman Model 21 Flamephotometer[2] was used for determination of sodium and potassium

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[1] Coleman Instruments, Inc., Maywood, Ill.
[2] Ibid.

in urine. The urine was suitably diluted to bring the readings within the range of the instrument. The procedure given in the Manual on Operating Directions for the Coleman Flame Photometer (1960) was followed, using appropriate filters and a direct reading scale. The results were expressed in milliequivalents per kilogram of body weight per day.

Ketosteroids in Urine. A method developed by Few (75) for fractionating the 11-oxy and 11-deoxy-ketosteroids in urine was adopted in the present experiment. Since the method was developed for human urine ketosteroid determination, a slight modification for the rabbit urine, which contains relatively more water, was introduced to bring the concentration so as to give optical densities which could be read in a spectrophotometer. For this purpose 40 ml. of urine were concentrated to about half the volume under vacuum at 37°C. The pH of the concentrated urine was adjusted to pH 7.0.

Two ml. of a freshly prepared reagent of 10 per cent solution of sodium borohydride in 0.1 N sodium hydroxide was added to the urine. Reduction was allowed to take place at room temperature for two hours. At the end of two hours 1 ml. of 25 per cent acetic acid was added drop by drop with gentle shaking to destroy excess borohydride. A few drops of diethyl ether were added to prevent frothing. After 15 minutes 8 ml. of a freshly prepared solution of sodium metaperiodate and 1.6 ml. of 1 N sodium hydroxide were added. The pH was checked again and adjusted to pH 6.5 to 7.0 if necessary. The mixture was incubated at 37° C. in a water bath for one hour, and then

0.5 ml. of 10 N sodium hydroxide were added and incubated for another 15 minutes. The mixture was then cooled under tap water for two hours.

The mixture was then extracted twice with diethyl ether, and the combined ether extracts were pooled and shaken in separatory funnels with 5 ml. of 5 per cent sodium dithionate in 10 per cent sodium hydroxide solution. The extract was then triple washed with distilled water. The clear ether layer was transferred to a porcelain dish and evaporated to dryness under a fan in a fume hood.

Celite 545 was treated overnight with three times its weight with 50 per cent hydrochloric acid. The celite was then washed with distilled water until free from acid and later dried in an oven at 110°C. for one hour.

Two hundred and seventy ml. of petroleum ether, b.p. 80° to 100°C., 30 ml. of benzene, 50 ml. of ethanol, and 50 ml. of water were shaken together and the layers allowed to separate out. The top layer was the mobile phase, and the bottom layer was the stationary phase.

One gram of celite 545 was mixed in sequence with 0.4, 0.4, and 0.2 ml. quantities of stationary phase and suspended in 15 ml. of mobile phase. The suspension was transferred to 10 x 300 ml. column tubes with a wide bore teat pipette. With a plunger the celite column was packed well so that the height of the column was 3.5 centimeters.

The residue in the porcelain dish was dissolved in 0.1 ml. of benzene and transferred to the column with two 1 ml. and two

0.5 ml. successive volumes of the mobile phase. A further quantity of 10 ml. of the mobile phase was added to the column. First an 8 ml. and then a 5 ml. fraction was collected separately into properly labelled tubes. The column was later developed with benzene, collecting 10 and 5 ml. fractions. The fractions were labelled A, B, C, and D, respectively. The fractions B and D were discarded, as they do not contain any Zimmerman reactive material. Fraction A contained 11-deoxy-17-oxosteroids, and fraction C contained 11-oxy-17-oxosteroids and were evaporated under vacuum at 37°C. in a vacuum drying oven.

The method of Kafka and Bondy (129) was followed for color development.

Two volumes of a 2 per cent solution of m-dinitrobenzene in ethanol, 3 volumes of 5 N sodiumhydroxide, and 2 volumes of 95 per cent ethanol were mixed together just before use as the m-dinitrobenzene reagent.

Equal volumes of amyl acetate and 75 per cent ethyl alcohol were mixed together just before use as the amyl acetate reagent.

To each of the tubes including a blank, 0.7 ml. of m-dinitrobenzene reagent was added, mixed and allowed to stand for 90 minutes in the dark. At the end of the period 2 ml. of amyl acetate reagent were added, shaken up, and allowed to stand in the dark for about 8 minutes. The supernatant solution was pipetted out into a Coleman cuvette and the readings for optical density were made at 510 millimicrons wavelength after adjusting to 100 per cent transmittance with the blank.

A standard curve was plotted with known quantities of etiocholanalone representing fraction A and 11-hydroxy-etiocholanalone representing fraction C. The values were expressed in mg. of ketosteroid per day per rabbit.

Sodium, Potassium, and Calcium in Serum. These estimations were made on a Coleman Model 21 Flame photometer following the instructions given in the Manual as was done in the determination of urinary sodium and potassium. For estimation of calcium the concentration of sodium was adjusted to 150 milliequivalents per liter. The results were expressed in milliequivalents per liter of serum.

Serum Magnesium Determination. A modified method of Orange and Rhein (182) as described in Lab-trol Manual of "Dade Reagents", Incorporated,[1] was followed.

Two ml. of the serum were pipetted into a 15 ml. centrifuge tube. To each of the tubes 4 ml. of distilled water and 2 ml. of trichloroacetic acid were added, mixed, and centrifuged for 20 minutes at 3,000 revolutions per minute (r.p.m.). Two ml. of the supernatant were pipetted into a small test tube, and 1 ml. of distilled water was added. To another tube 1 ml. of working standard solution of magnesium sulfate containing 0.015 mg. of magnesium per 1 ml. was pipetted out, and 1.5 ml. of distilled water and 0.5 ml. of 10 per cent trichloroacetic acid were added.

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[1]Dade Reagents, Inc., Miami, Florida.

A blank was prepared containing 2.5 ml. of distilled water and 0.5 ml. of 10 per cent trichloroacetic acid solution.

One ml. of 0.1 per cent polyvinyl alcohol solution in water, 0.035 per cent titan yellow working solution, and 16 per cent sodium hydroxide solution were added to the unknown, standard, and blank tubes.

The color developed was measured in a Colsman Model 14 Spectrophotometer at 560 millimicrons wavelength after adjusting to 100 per cent transmittancy with the blank.

$$\text{mg. of magnesium per 100 ml. of serum} = \frac{\text{density of unknown}}{\text{density of standard}} \times 3$$

The results were expressed in milliequivalents per liter of serum.

Serum Cholesterol Determination. Free and total cholesterol in serum were determined by the method of Ferro and Ham (74).

Into a 15 ml. graduated centrifuge tube 0.5 ml. of serum was pipetted and 4.5 ml. of isopropyl alcohol added, stoppered, shaken for a few seconds and allowed to stand for 15 to 20 minutes. Each tube was shaken again, stoppers were removed, and the mixture was centrifuged at 1500 r.p.m. for 10 minutes.

For total cholesterol determination, 1 ml. of the supernatant was pipetted into a 15 ml. centrifuge tube. To each tube 0.5 ml. of alcoholic potassium hydroxide was added, mixed by swirling, and placed in a water bath at 37°C. for 30 minutes. A small drop of phenolphthalein indicator solution was added to

each tube and titrated carefully with 10 per cent acetic acid until the pink color disappeared. Finally a small drop of acetic acid solution was added in excess. One ml. of 1 per cent digitonin in ethyl alcohol and 1 drop of 30 per cent aluminum chloride were added, mixed, and allowed to stand for 30 minutes at room temperature. The tubes were then centrifuged for 10 minutes at 3,000 to 3,500 r.p.m., and the supernatant was drained by inverting the tubes rapidly on a paper towel for a few minutes. The precipitate was then washed with 3 ml. of acetone and centrifuged again. The acetone was drained off carefully, and the precipitate was shaken up with 0.2 ml. of distilled water by tapping the bottom of the tube against the palm of the hand.

For free cholesterol determination 2 ml. of the supernatant were pipetted into a graduated centrifuge tube. One ml. of digitonin and a drop of aluminum chloride solution were added. The rest of the procedure followed was the same as for total cholesterol.

From the working standard solution containing 0.2 mg. per ml. of cholesterol in isopropyl alcohol, 0.5, 1.0, and 2.0 ml., respectively, were pipetted into 3 separate test tubes and used as the standard. From this stage digitonin solution was added to each, and the procedure for free cholesterol estimation was followed.

To each of the total, free, and standard cholesterol tubes 6 ml. of the color development mixture were placed directly on the precipitate. The color development mixture was prepared by mixing 20 ml. of a mixture of 3 volumes of acetic anhydride and

2 volumes of glacial acetic acid, which was kept as a stock mixture. Twenty ml. of the stock mixture and 2 ml. of concentrated sulfuric acid were mixed and cooled to room temperature. This mixture was prepared on the day of its use.

The mixture from the centrifuge tubes was transferred to cuvette 90 seconds after the addition of color development mixture, and readings were taken at 640 millimicrons wavelength after adjusting to 100 per cent transmittance with distilled water.

$$\text{Total cholesterol in mg. per 100 ml.} = \frac{\text{density of unknown}}{\text{density of standard}} \times \text{value of standard.}$$

$$\text{Free cholesterol in mg. per 100 ml.} = \frac{\text{density of unknown}}{\text{density of standard}} \times \frac{\text{value of standard}}{2}$$

Serum Transaminases. Serum glutamic oxalacetic transaminase and serum glutamic pyruvic transaminase concentrations were determined by the Reitman Frankel (207) method as given in "Dade Reagent" brochure. The enzyme glutamic oxalacetic transaminase catalyzes the conversion of asparatic acid and alpha-ketogluteric acid to glutamic acid and oxalacetic acid. The ketoacids formed were reacted with dinitrophenylhydrazine to form ketoacid hydrazones which, upon addition of sodium hydroxide, yielded an intense brownish color that was read in a Coleman Model 14 Spectrophotometer and compared with a standard curve drawn with known quantities of ketoacids.

The conversion of alanine and alpha-ketoglutaric acid to glutamic acid and pyruvic acid, which is catalyzed by glutamic

pyruvic transaminase, was estimated by developing a color with dinitrophenyl hydrazine, similar to that of glutamic oxalacetic transaminase.

The results were expressed in Frankel units per ml. of serum.

Ubiquinone in Liver, Muscle, and Adrenal Gland. The method described by Moore and Sharman (166) was adopted.

Small pieces of liver or skeletal muscle weighing about a gram and whole adrenal glands were lightly blotted to remove excess moisture, weighed, and finely ground in a small porcelain basin. The minced tissue was digested in a mixture of 0.5 ml. of 65 per cent aqueous solution of potassium hydroxide and 2 ml. of ethanol by heating on a water bath until vapors of alcohol were noticed. Then 0.5 ml. of alcoholic solution of pyrogallol was added and digested for 3 minutes with occasional shaking. Five ml. of distilled water were added to the digest and transferred to a separatory funnel. The digest was extracted with 10 ml. quantities of ether. The combined extracts were washed twice with distilled water, transferred to a test tube and evaporated to dryness under reduced pressure in a vacuum drying oven at 37°C.

The residue was then dissolved in 5 ml. of cyclohexane. The absorption was measured in a Beckman Model Du Spectrophotometer[1] at 272 millimicrons. Corrections for vitamin A as described by Moore and Sharman (166) were used.

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[1]Beckman Instruments, Inc., Fullerton, California.

A standard curve was plotted with known quantities of ubiquinone, and the absorbancies of the unknown were converted to micrograms per gram of fresh tissue.

Manometric Technique with Tissue Slices. Respiration studies on skeletal and cardiac muscle were made by Warburg's constant volume technique, as described by Umbreit et al. (217). The respiration studies were carried out on various tissues of all rabbit groups.

The rabbits were sacrificed by cardiac bleeding and stunning. Immediately thereafter the semitendinosus muscle and heart were transferred into oxygenated Krebs' Ringer phosphate buffer solution of pH 7.4, 37°C. Employing corneal scissors and forceps, muscle strips of approximately half a millimeter in thickness and 1 centimeter in length were peeled and transferred to the oxygenated Krebs' Ringer phosphate solution[1] at 37°C., as described by Richardson, Shorr, and Loebel (183). Cardiac slices half a millimeter in thickness were prepared with a Stadie-Riggs tissue slicer.[2] The muscle strips and cardiac slices were blotted and weighed in a "Right-A-Weigh" balance.[3] The muscle strips weighed 90 to 130 mg. and cardiac muscle slices 45 to 70 mg. The slices and strips were quickly transferred to Warburg's respiration flasks containing 2.3 ml. of Krebs' Ringer phosphate solution, with or without alpha-tocopherol and/or sodium selenite. The systems were then closed

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[1]Described by Umbreit et al. (217).
[2]Arthur H. Thomas Co., Philadelphia, Pa., U.S.A.
[3]Wm. Ainsworth & Sons, Inc., Denver, Colo., U.S.A.

to the atmosphere, and the manometer units were then set in the water bath at 37°C. and shaken at 100 oscillations per minute. The entire system was allowed to equilibrate for 30 minutes. Then the stopper was quickly opened, the level of fluid adjusted to 150 mm. and the stopper was closed. The oxygen consumption was recorded over a period of 1 hour taking readings at 15-minute intervals. The thermobarometer was set initially at 150 mm. and was used for correction of temperature and pressure changes.

The effect of *in vitro* addition of alpha-tocopherol and selenium was studied as compared to the negative and positive controls. Alpha-tocopherol was emulsified in Krebs' Ringer phosphate solution to a final concentration of 5 mg. per cent. Selenium was added as sodium selenite to a final concentration of 0.10 parts per million in Krebs' Ringer phosphate solution.

The tissue slices or strips were set in duplicate in

(1) Krebs' Ringer phosphate solution;

(2) Krebs' Ringer phosphate solution with alpha-tocopherol;

(3) Krebs' Ringer phosphate with selenite;

(4) Krebs' Ringer phosphate with alpha-tocopherol and selenite selenium.

The tissue dry weights were determined by drying duplicate portions of tissues in an oven at 110°C. for 24 hours and the dry weight calculated from the actual loss in weight. The oxygen consumption was expressed as microliters of oxygen per mg. of dry tissue per hour.

Packed Cell Volume. Blood was drawn by ear vein puncturing and collected directly into a heparinized capillary tube of 1.2

to 1.4 ml. diameter and 75 ml. length. One end of the tube was sealed with Clay Adams' Sealease wax[1] and spun in a Clay Adams microhaematocrit centrifuge[1] for 10 minutes. The packed cell volumes were read in an Adams Microhaematocrit[1] reader and expressed as a percentage.

Total Leucocyte Count. Blood was drawn directly into a heparinized capillary tube from the punctured ear vein and placed on a microscopic slide. Twenty microliters of this blood were drawn into a white cell pipette and discharged into a tube containing 10 ml. of normal saline. One-tenth ml. of saponin solution was added to each tube, and the tubes were allowed to stand for 10 minutes to promote red blood cell lysis. The tube was then transferred to Coulter Counter[2] and the number of white cells recorded and correction made for the background count taken with the blank containing normal saline and saponin.

Differential Leucocyte Count. A thin blood smear was made, fixed in methyl alcohol, and stained with Wright's stain. After drying, differential leucocyte counts were determined under an oil immersion lens of a microscope by counting a minimum of 200 cells. The results were expressed as percentages of the total leucocyte count.

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[1]Clay Adams, Inc., New York.
[2]Coulter Electronics, Kenmore, Chicago, Ill.

RESULTS AND DISCUSSION

Effect of Avitaminoses A and/or E on Food and Water Consumption, Urine Volume, Growth Rate, Urinary Creatine/Creatinine Ratio, Serum Transaminase Levels and Post Mortem Changes

Results.

(a) Avitaminosis A. The growth rate was relatively better in vitamin A-deficisnt group as svident from the data presented in Table 2 and Fig. 1. The animals of this group continued to grow steadily until the eighth week but showed a decline thersafter. There was a slight depression of growth during the fourth week which may have been due to a slight off flavor developed by ths feed during storage when the animals did not eat the ration readily. In ths present experiment, xerophthalmis did not develop even by the tenth week on the vitamin A-deficient diet. Howevsr, the vitamin A-deprived rabbits showed a sluggish light reflex when tssted with a flashlight. Therefore, decline in growth rate and sluggish light reflex were taken as early indications of deficiency, and the animals wers sacrificed to collect tissues for further studies.

Ths dates of sacrifice of each animal are presented below:

A-1 (Male) - 7/2/64

A-2 (Female) - 6/26/64

A-3 (Mals) - 7/4/64

A-4 (Female) - Died from bleeding after cardiac puncture on 6/24/64.

The reason for ths sarly sacrifice of ths animals before ssvere signs of dsficiency wers manifest was to compare the

biochemical and tissue changes during an identical period with vitamin E-deficient and A and E-deficient animals in which visible changes of deficiency were evident.

Water and feed consumption fluctuated slightly during the course of the experiment. However, a tendency to decrease towards the end was evident from the data presented in Table 1.

Creatine and creatinine levels were not affected significantly and were comparable to that of the control animals and are presented in Table 3 and Fig. 2.

Serum glutamic oxalacetic transaminase and glutamic pyruvic transaminase levels were not altered much, although there was a slight rise in the glutamic oxalacetic transaminase by the thirtieth day and the glutamic pyruvic transaminase by the forty-fifth day. The levels of these enzymes in the serum dropped to the basal level during subsequent weeks.

Visible lesions were not evident at post mortem examination except in animal number A-2, which showed oocysts of coccidia in the feces and presented two yellow spots on the liver which might have resulted from infection with coccidia. This particular animal had shown diarrhea and considerable loss of weight.

(b) Avitaminosis E. The animals of this group reached their maximum rate of gain by the sixth week after which the growth curve showed a plateau with a slight decline, as shown in Table 2 and Fig. 1. About the sixth week the urine volume tended to decrease, and the water intake was proportional to the urinary output. The urine volume decreased about 30 per

Table 1. Average volume urine[1] excreted, water[2] and feed[3] consumed at weekly intervals.

Group	Weeks										
	0	1	2	3	4	5	6	7	8	9	10
A deprived											
Urine	214.5	308.0	195.0	166.0	158.0	265.0	142.5	153.0	159.7	117.0	189.0
Water	381.0	245.0	190.0	250.0	302.5	172.5	196.0	227.5	183.8	300.0	
Feed	36.0	36.0	20.0	23.5	28.5	32.0	28.25	24.0	23.75	36.0	
E deprived											
Urine	154.0	195.0	158.0	173.5	128.0	167.5	116.0	103.0	102.3	78.3	-
Water	248.0	305.0	235.0	278.0	245.0	150.0	187.0	163.3	146.7	-	
Feed	58.25	36.0	19.2	31.3	34.25	28.5	26.0	22.7	26.33		
A and E deprived											
Urine	183.0	242.5	234.3	267.0	263.8	202.5	203.0	119.0	123.0	180.0	-
Water	280.0	238.5	357.0	350.0	262.5	249.0	153.0	100	160.0		
Feed	44.25	32.8	23.5	25.0	25.5	31.25	23.0	17.5	18.0		
Control											
Urine	189.0	234.0	198.0	196.0	233.3	165.0	236.0	218.0	117.5		-
Water	316.0	195.0	173.0	193.0	253.3	323.0	320.0	250.0	97.5		
Feed	37.0	28.0	20.0	21.3	32.3	26.0	33.0	37.5	18.5		

[1]Collected one day per week and measured in ml. per day.
[2]Recorded one day per week and measured in ml. per day.
[3]Recorded one day per week and measured in grams per day.

Table 2. Average body weights[1] of rabbits at weekly intervals.

Group	Number of animals	Weeks										
		0	1	2	3	4	5	6	7	8	9	10
Vitamin A-deprived	4	1208	1293	1442	1732	1756	1829	1910	2033	2184	2179	2094
Vitamin E-deprived	4	1110	1146	1281	1416	1536	1586	1571	1800*	1803	1723	
Vitamins A & E deprived	4	1065	1212	1336	1483	1533	1616	1636	1623	1629	-	
Control	4	1061	1140	1262	1393	1413	1543	1620	1686	1777	1819	-

[1]Weighed once weekly and measured in g. per day.
*Average of three animals only as one was destroyed.

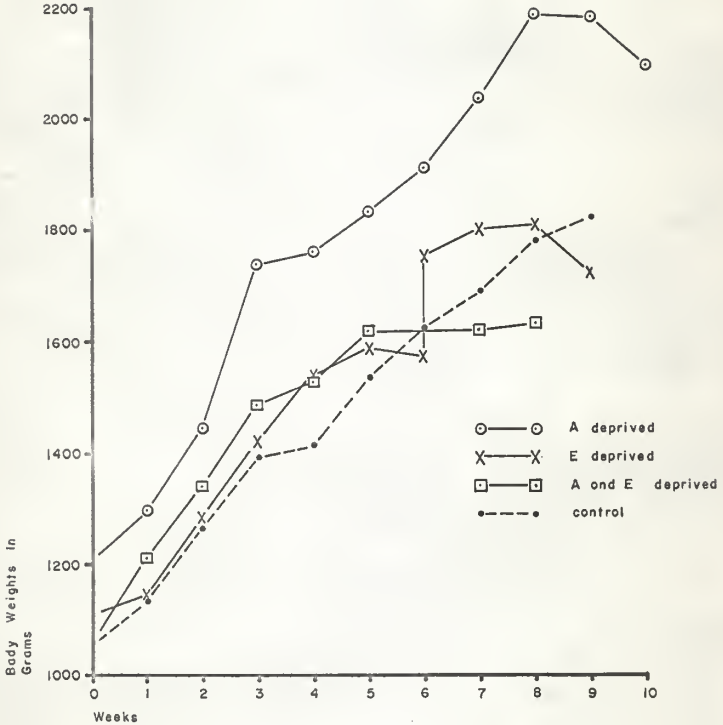

Fig. 1 Variations in body weights ot weekly intervols of vitamin A ond/or E deprived rabbits compored to controls.

Table 3. Average creatinine[1] and creatine[2] levels in the urine at weekly intervals.

Group	Weeks										
	0	1	2	3	4	5	6	7	8	9	10
A deprived											
Creatinine	52.00	16.00	34.0	39.84	58.00	38.50	51.20	72.50	67.00	76.50	62.50
Creatine	0.50	0.00	0.00	0.00	1.50	1.00	2.80	2.00	8.00	16.50	0.00
Ratio	0.00	0.00	0.00	0.00	2.60	2.60	5.50	2.76	10.66	21.56	0.00
E deprived											
Creatinine	50.00	14.00	31.5	32.10	36.50	29.34	41.25	22.5	61.50		
Creatine	5.50	0.00	2.5	3.57	6.00	6.30	30.25	17.0	16.50		
Ratio	11.00	0.00	7.9	11.00	16.44	21.12	73.40	75.33	26.83		
A & E deprived											
Creatinine	50.00	17.00	35.00	56.07	33.00	55.00	18.50	22.50	53.00	42.00	
Creatine	5.50	0.020	8.00	5.34	1.50	6.50	24.50	20.25	22.50	30.50	
Ratio	11.00	0.08	7.84	9.50	4.54	11.80	132.40	90.00	41.51	72.61	
Control											
Creatinine	53.00	23.00	35.00	37.04	37.50	9.00	35.50	16.60	59.00	152.00	
Creatine	0.50	0.00	0.00	0.00	0.00	1.00	4.00	0.05	4.00	5.00	
Ratio	1.13	0.00	0.00	0.00	0.00	11.10	11.20	0.30	6.95	3.29	

1 and 2 Milligrams per 100 ml. urine.

Fig. II Variations in creatine / creatinine ratios of vitamin A and/or E deprived rabbits at weekly intervals compared to controls.

Table 4. Serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase values[1] at fifteen-day intervals.

Group	Days				
	0	15	30	45	60
A deprived					
SGOT	42.0	46.6	76.0	34.0	29.5
SGPT	21.0	20.0	21.0	50.0	19.0
E deprived					
SGOT	45.0	45.2	100.0	100.0	84.0
SGPT	36.0	19.0	46.0	86.0	117.0
A & E deprived					
SGOT	50.6	44.2	38.0	84.0	133.0
SGPT	16.0	58.0	24.0	250.0	260.0
Control					
SGOT	44.4	45.2	44.0	34.0	36.0
SGPT	36.0	37.2	38.0	32.0	35.0

[1]Reitman Frankel units.

Reitman Frankel unit = amount of enzyme which will cause at 25°C. and 340 mu a decrease in optical density of 0.001 per minute.

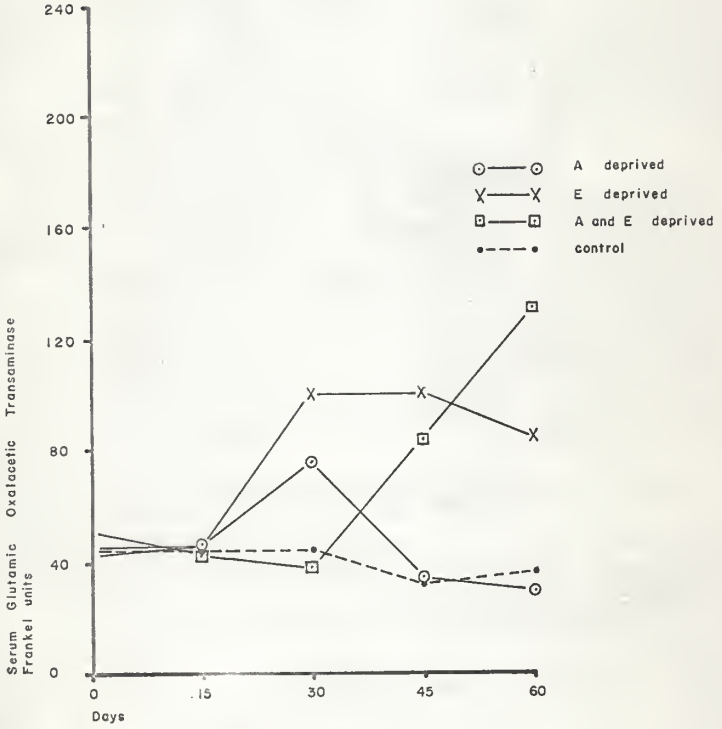

Fig. III Variations in serum glutamic oxalacetic transaminase levels of vitamins A and/or E deprived rabbits compared to controls at 15 day intervals.

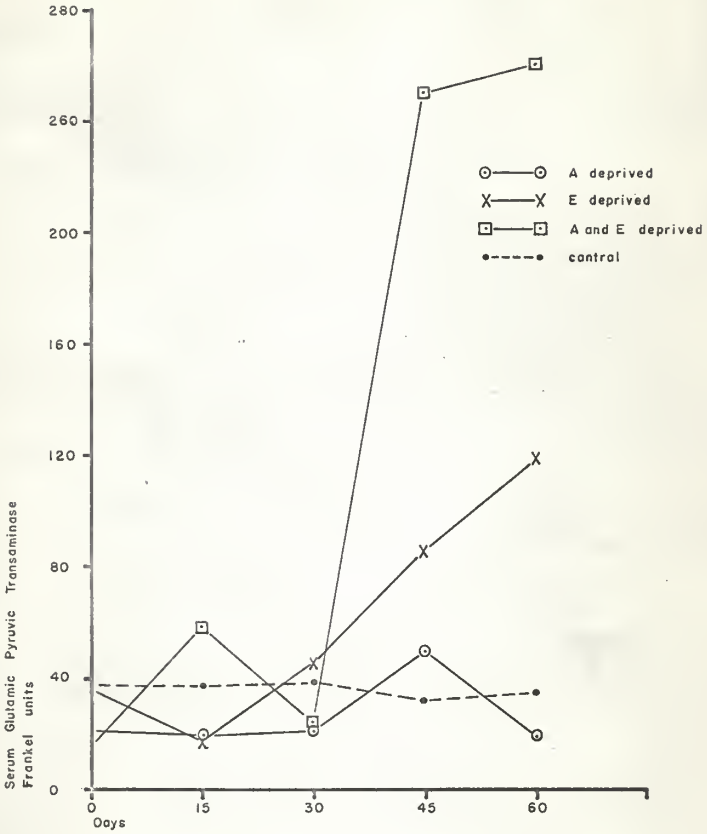

Fig. IV Variations in serum glutamic pyruvic transaminase levels of vitamins A and/or E deprived rabbits compared to controls at 15 day intervals.

cent during the eighth week. The food intake gradually declined from the second week.

A week prior to the time when maximum body weights were reached, the urinary creatine levels began to increase rapidly. The creatine/creatinine ratio exceeded 70, which was taken as an indication of onset of muscular dystrophy. This ratio was maintained for another week and decreased again during the eighth week.

The serum glutamic oxalacetic transaminase levels were more than doubled by the thirtieth day and maintained until the end. These results, presented in Table 4 and Figs. 3 and 4, show that the serum glutamic oxalacetic transaminase levels began to rise even before the creatine levels showed a great increase. However, serum glutamic pyruvic transaminase concentration increased steadily from the fifteenth day onward, as delineated in Fig. 4.

The rabbits of this group were sacrificed when it was certain that muscular dystrophy had appeared as evident from the indifference of these animals to move when disturbed and by the indication given by creatine/creatinine ratio. These animals were not able to raise themselves up when held by their hind legs. One of the animals, E-3, demonstrated signs of paralysis of the left foreleg by the fifth week, was not able to move or right herself when placed on her side, consumed very little food and water, and excreted very little urine even during attempts to press the bladder. These animals were sacrificed on the following dates:

E-1 (Male) - 6/27/64
E-2 (Female) - 6/25/64
E-3 (Male) - 6/3/64
E-4 (Female) - 6/29/64.

On post mortem examination the skeletal muscles, particularly of the hind limbs, were flaccid and showed white streaks. In the case of one animal, E-2, the muscles appeared marbled instead of presenting white streaks over a pale background. The heart muscle did not demonstrate any visible abnormality except that it was flabby and pale in appearance. In most cases some fibrosis appeared where the heart was punctured for cardiac bleeding. No visible changes were noticed in other organs. Animal E-4 exhibited a few ova of coccidia in the cecal contents but never showed any diarrhea.

(c) Avitaminosis A and E. In the group of animals where vitamin A-deficiency was superimposed on a vitamin E-deficiency, the growth rate reached a plateau by the fifth week and did not change during the rest of the period.

The urine volume and feed and water consumption decreased from the sixth week. The creatine/creatinine ratio showed a slight increase by the fifth week and made a sudden peak by the sixth week, but dropped again about the tenth week. However, the creatine excretion remained high.

The serum glutamic oxalacetic transaminase levels increased steadily from the thirtieth day and almost tripled the initial level by the sixtieth day. The serum glutamic pyruvic transaminase concentration was markedly elevated by the forty-fifth

day, reaching a level almost eight times that of the initial levels, as delineated in Fig. 4.

Animals of this group were sacrificed when the body weight decreased and showed symptoms of muscular dystrophy as evidenced by reluctance to move when forced. None of these animals showed evidence of xerophthalmia, although the light reflex was sluggish.

The muscles were pale and had white streaks. In some cases the liver was enlarged and showed either a parboiled or friable appearance with white foci scattered all over the surface. The gall bladder was distended in all cases except AE-1 where it was normal. The heart muscle appeared pale and flabby, and no microscopic changes could be noticed.

The animals were sacrificed on the following dates:

AE-1 (Female) - 6/19/64
AE-2 (Male) - Died on 6/18/64
AE-3 (Male) - 6/15/64
AE-4 (Female) - 6/30/64

Discussion. From a perusal of the above results one observes that the onset of induced vitamin A-deficiency is slow to appear. In the present investigations the deficiency did not progress to the extent of exhibiting symptoms of xerophthalmia, which is the most pathognomonic sign of avitaminosis A. The state of deprivation of vitamin A was at an early stage showing only depression of growth rate and a sluggish light reflex. Similar signs were described in rabbits in early stages of vitamin A-deficiency by Phillips and Bockstedt (190). Since the

animals in this experiment were receiving alpha-tocopherol acetate, the slow and insidious onset might be due to the protective action of vitamin E on vitamin A. That vitamin E protects vitamin A by preventing oxidative destruction or by protecting it by some other mechanism was reported by Cox et al. (41). It is interesting to note the observation of Chevrel and Cornier (35) who reported that vitamin A-deficiency symptoms in rabbit would not appear until and unless the animals were simultaneously deficient in vitamin E. Further findings of Hickman et al. (107) demonstrated that small amounts of vitamin E increased the growth promoting action of vitamin A and lengthened the survival of these animals. It can be concluded from the above that depletion of vitamin A and its destruction would be low in the presence of small quantities of vitamin E. Probably vitamin E may bring about an economic utilization of stored vitamin A.

Lesions in liver and kidney were not noticed in the present studies, although they were observed by Phillips and Bockstedt (190). These findings are correlated to the levels of serum glutamic pyruvic transaminase, which should naturally be elevated had there been any damage to the liver or kidney. Even this finding would show that the deficiency had not progressed towards the advanced stage.

From these studies with rabbits, vitamin A does not appear to be concerned with creatine metabolism in the muscles, since the creatine levels in urine remained low throughout the experiment.

The results of these investigations on avitaminosis E agree

well with the findings of Mackenzie and McCollum (144), who reported that creatinuria actually precedes the growth depression by 18 days. A creatine/creatinine ratio of 70 and the decline in growth rate occurred on about the sixth and seventh weeks, respectively. In the case of one animal these changes occurred a week earlier. The symptoms presented by these animals at the time they were sacrificed were of the second stage as described by Mackenzie and McCollum (144). However, this stage seemed to last longer than three and a half days. Even the onset of dystrophic symptoms appeared to begin a little later than that observed by Mackenzie and McCollum (144) and Borgman (21). The rapidity and degree of severity of the lesions in these experiments would suggest that the cod liver oil, when added to the diet, probably acts as a stressing agent. Borgman (21) inferred from his results that cod liver oil in the diet or cortisone injections increased the severity of lesions. The present studies, using linoleic and linolenic acids in the place of cod liver oil, as was adapted by Borgman (21), produced the same results. A factor which affects the time for attaining maximum weight may be the initial weight or age of the animals when they are started on the dietary regimen. In an experiment using 500 gram weight rabbits, Morgulis and Spencer (167) reported that the maximum weight was reached by 26 days, whereas with larger rabbits maximum weight was attained in 37 days. The results of the present experiments revealed that maximum growth on the deficient diets was reached by about six or seven weeks when rabbits of average initial body weight of 1,098 grams were used.

A 50 per cent decrease in feed consumption agrees with the findings of Mackenzie and McCollum (144), and the decline in urine volume at the sixth week agrees with the observations of Morgulis and Spencer (167). However, in the case of one rabbit which reached stage three, volume of urine did not increase.

The increase in serum glutamic oxalacetic transaminase levels is in agreement with what was reported in lambs and calves by Blincoe and Dye (18), Kutler and Marble (134), Swingle et al. (235), and Blincoe and Marble (19). However, the elevation of serum glutamic oxalacetic transaminase levels was not as great as was reported by the above workers. Inasmuch as the level of this enzyme rose from the fifteenth day onward, this parameter could be taken as an index of onset of dystrophy, as was suggested by Blincoe and Marble (19) and Swingle et al. (235). No information was available on the changes in serum glutamic pyruvic transaminase levels. In the present investigations it was observed that the glutamic pyruvic transaminase levels steadily increased from the fifteenth day on. Elevated levels of this enzyme in serum have been found by many workers (106, 260, 261) in liver and kidney diseases in human beings as well as in animals. Although liver and kidney have not been reported to be affected in rabbits, necrosis of liver has been described as a predominant manifestation of avitaminosis E in rats where muscular dystrophy was not extensive. The present findings indicate the possibility of latent liver or kidney damage not apparent to the naked eye or under the microscope. Although it can be postulated that a wide variety of tissues are affected in

various species, the predominant lesion is found in one or the other of the tissues.

In the present investigations on avitaminoses A and E, both serum glutamic oxalacetic and glutamic pyruvic transaminase levels increased although the latter was elevated markedly after the thirtieth day. Also substantiating this observation, the liver of most of the animals showed gross lesions. In this group of animals growth rate declined about the seventh week and creatinuria was marked during the sixth week, which was similar to that of simple vitamin E-deficiency. The rabbits appeared healthy and grew normally till about the sixth week when a sudden adverse change took place. Even in this group the elevation of transaminases was the earliest change noticed. The creatine/creatinine ratio was higher than in the case of simple avitaminosis E. Butturuni (30) reported a marked synergism between vitamin A and E in reducing creatinuria in rats, and this probably explains the reason for a high creatinuria in rabbits in a combined deficiency of vitamins A and E. Although Edwin (66) reported a longer survival time of rats when deprived of both vitamins A and E, the present investigations show no difference, at least in the case of rabbits. In the case of one animal on vitamin A and E deficient diet, the adverse change suddenly occurred and the animal succumbed without adequate premonitary symptoms.

Effect of Avitaminoses A and/or E on the Urinary Ketosteroids and the Concentration of Cations in Urine and Serum

Results. The Zimmerman reactive urinary ketosteroids were fractionated by column chromatography into the 11-deoxy-17-oxosteroids, and 11-oxy-17-oxosteroids. Androsterone and etiocholanalone represent the metabolic excretory products of testosterone, Δ^4-androstene-3, 17-dione and dehydroepiandrosterone of adrenal cortical origin. The 11-oxy-17-oxosteroids, namely, 11-ketoandrosterone, 11-ketoetiocholanalone, 11-B-hydroxyandrosterone, 11-B-hydroxyetiocholanalone, represent the metabolic derivatives of cortisone and cortisol. The levels of these two fractions have been expressed as mg. per day per rabbit, as presented in Table 7 and Fig. 6.

The urinary sodium and potassium concentrations in urine were expressed in milliequivalents per kilogram of body weight per day, so that the levels between groups could be compared on the basis of unit body weight, rather than per animal per diem. The data for the different treatments are presented in Table 5 and Fig. 5. The levels of sodium, potassium, calcium, and magnesium concentration in serum are expresaed in milliequivalents per liter, and are given in Table 6.

In order to correlate the findings with regard to ketosteroids, the weights of adrenal glands were expressed in terms of per cent body weight as shown in Table 8.

Avitaminosis A. A perusal of the data presented in Table 7 shows that 11-deoxy-17-oxosteroids and 11-oxy-17-oxosteroids

decreased considerably in avitaminosis A. The adrenal glands were 0.0073 per cent of the body weight as compared to the controls which were 0.00937 per cent. The decrease in weight of the adrenals in avitaminosis A appears significant.

The sodium, potassium, calcium, and magnesium levels in serum did not reveal any significant trend, as the concentrations remained within the normal ranges. Urinary sodium and potassium levels recorded a fall during the first two or three weeks in all the groups concerned. Subsequent to the third week the levels fluctuated a little but were not altered greatly. Neither the serum magnesium nor calcium showed any significant chsnges in their concentrations.

Avitaminosis E. The levels of urinary 11-deoxy-17-oxosteroids decreased till about the fifth week, when a sudden increase occurred and was maintained until the end of the eighth week. However, by the end of the ninth week the levels once again were depressed to almost the levels at the end of the fifth week, as shown in Fig. 6. In contrast to what was observed with the 11-deoxy fraction, the 11-oxy-17-oxosteroids declined in concentration gradually from the beginning to the end of the experimental period. Regarding the weights of the adrenal glands in avitaminosis E, they averaged 0.0088 per cent of the body weight as against 0.00937 per cent in controls as presented in Table 8. The difference in weights of adrenals does not appear to be significant. However, in two animals, E-3 and E-4, the adrenals were enlarged.

Until the end of the eighth week the urinary sodium and

Table 5. Sodium and potassium concentrations in urine at weekly intervals.[1]

Group	0 Time		1 Week		2 Weeks		3 Weeks		4 Weeks		5 Weeks	
	Sodium	Potassium	Sodium	Potassium	Sodium	Potassium	Sodium	Potassium	Sodium	Potassium	Sodium	Potassium
A deprived	5.20	8.78	2.74	4.34	1.86	4.22	1.30	1.80	2.16	4.73	4.17	3.02
E deprived	3.14	8.06	2.97	4.90	2.77	4.83	2.07	3.67	0.87	2.23	1.06	3.38
A and E deprived	4.66	7.24	2.10	5.60	1.01	4.28	1.90	3.38	2.44	3.96	1.56	4.86
Control	4.67	6.06	3.30	3.90	2.49	3.45	1.80	1.42	2.57	5.03	1.88	2.84

[1]Collected once a week and measured in milliequivalents per kilogram of body weight per day.

Table 5 (Concl.). Sodium and potassium concentrations in urine at weekly intervals.[1]

Group	6 Weeks		7 Weeks		8 Weeks		9 Weeks		10 Weeks	
	Sodium	Potassium	Sodium	Potassium	Sodium	Potassium	Sodium	Potassium	Sodium	Potassium
A deprived	1.44	4.60	1.07	3.71	2.16	1.46	1.21	3.91	2.89	4.33
E deprived	0.86	2.12	0.50	1.60	0.67	1.76	1.33	6.24	-	-
A and E deprived	1.08	3.00	0.89	2.12	1.85	2.29	3.67	7.43	-	-
Control	2.06	2.24	0.83	4.34	1.06	2.00	0.98	1.58	-	-

[1]Collected once a week and measured in milliequivalents per kilogram of body weight per day.

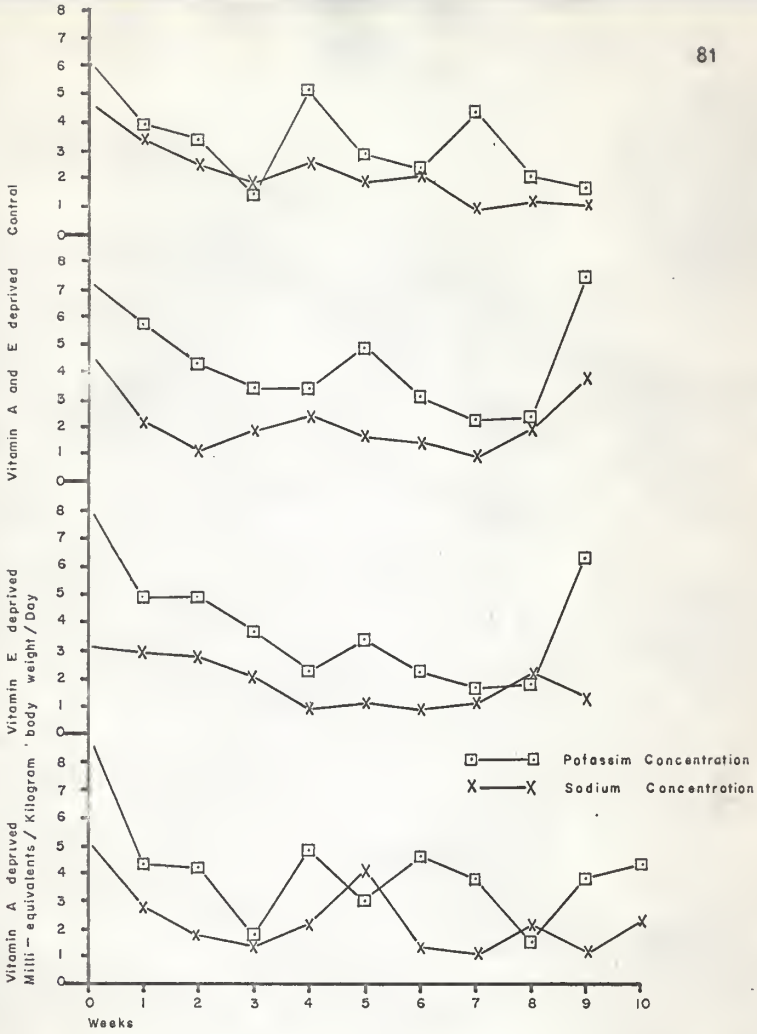

Fig. V. Variations in the rates of sodium and potassium excretion in urine at weekly intervals in vitamins A and/or E deprived rabbits at weekly intervals compared to controls

Table 6. Sodium, potassium, calcium, and magnesium concentrations[1] in serum at 15-day intervals.

Group	Days					
	0	15	30	45	60	75
A deprived						
Sodium	145.00	165.00	130.00	140.00	186.00	-
Potassium	3.55	4.60	3.85	4.10	3.90	-
Calcium	9.20	9.60	6.20	6.90	7.80	-
Magnesium	2.47	3.04	5.59	3.70	4.20	-
E deprived						
Sodium	148.00	218.00	228.00	280.00	156.00	-
Potassium	5.10	3.20	4.10	6.10	4.63	-
Calcium	7.50	9.20	6.35	6.25	7.60	-
Magnesium	2.33	2.88	3.19	3.78	3.58	-
A and E deprived						
Sodium	138.60	165.00	145.00	145.00	145.70	-
Potassium	4.25	6.75	3.70	7.10	5.23	-
Calcium	7.20	7.40	6.65	6.20	7.70	-
Magnesium	2.23	2.63	2.79	3.91	3.10	-
Control						
Sodium	152.00	280.00	135.00	200.00	135.00	145.50
Potassium	5.10	5.20	4.10	4.00	4.10	4.65
Calcium	6.20	4.90	7.30	7.60	6.95	8.45
Magnesium	2.55	3.12	3.17	2.82	4.57	-

[1] Milliequivalents per liter.

Table 7. Urinary ketosteroid[1] levels at weekly intervals.

Group	Weeks										
	0	1	2	3	4	5	6	7	8	9	10
A deprived											
11-deoxy-17-oxo-steroids	0.081	0.069	0.118	0.065	0.055	0.077	0.048	0.020	0.023	0.029	0.024
11-oxy-17-oxo-steroids	0.080	0.050	0.081	0.062	0.061	0.094	0.052	0.053	0.054	0.034	0.039
E deprived											
11-deoxy-17-oxo-steroids	0.063	0.065	0.037	0.041	0.026	0.024	0.068	0.058	0.055	0.023	
11-oxy-17-oxo-steroids	0.085	0.058	0.020	0.027	0.050	0.022	0.027	0.022	0.016	0.012	
A & E deprived											
11-deoxy-17-oxo-steroids	0.034	0.040	0.035	0.029	0.033	0.030	0.020	0.035	0.015	0.018	
11-oxy-17-oxo-steroids	0.029	0.028	0.024	0.027	0.030	0.021	0.018	0.028	0.036	0.034	
Control											
11-deoxy-17-oxo-steroids	0.031	0.039	0.044	0.036	0.032	0.038	0.055	0.051	0.035	0.030	
11-oxy-17-oxo-steroids	0.037	0.051	0.045	0.032	0.036	0.029	0.057	0.046	0.036	0.038	

[1] Collected once a week and measured in milligrams per day per rabbit.

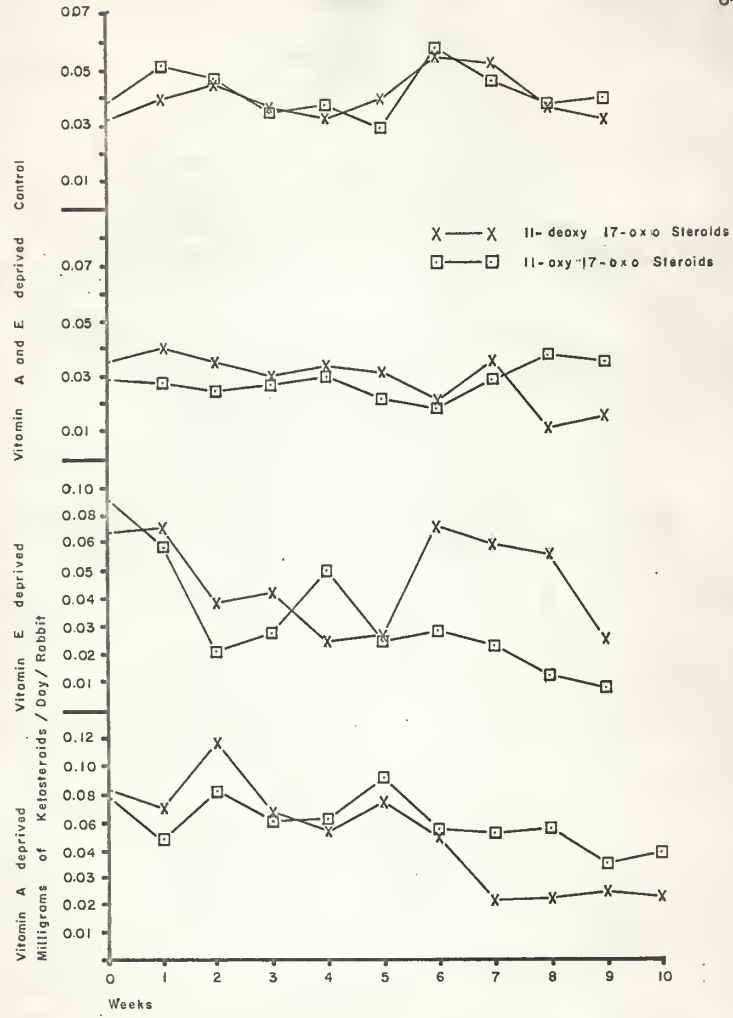

Fig. VI Variation in the rates of urinary 11-oxy and 11-deoxy, 17 ketosteroids excreted in urine by vitamins A and/or E deprived rabbits at weekly intervals compared to controls.

Table 8. Average weights of adrenal glands expressed as per cent of body weight.

Group	Average weight of adrenal glands in milligrams	Weight of animal at the time of sacrifice in grams	Weight of adrenal as per cent of body weight
A deprived			
A-1	48.5	2216	0.0044
A-2	97.0	2530	0.0077
A-3	78.7	1536	0.0102
A-4	83.4	2340	0.0070
Mean	76.9	--	0.0073
B deprived			
B-1	41.0	1936	0.0042
B-2	64.0	1696	0.0075
B-3	79.1	1028	0.0130
B-4	70.0	1536	0.0103
Mean	58.5	--	0.0088
A & B deprived			
AB-1	102.2	1756	0.0117
AB-2	86.5	1536	0.0046
AB-3	80.0	1610	0.0099
AB-4	126.7	1596	0.0160
Mean	78.9	--	0.01055
Control			
C-2	73.8	1552	0.0095
C-3	122.9	2086	0.0118
C-4	57.3	1676	0.0068
Mean	84.7		0.00937

potassium levels did not show any changes, but by the end of the ninth week the potassium concentration increased and sodium decreased. An increase in serum sodium concentration was noticed from the fifteenth to the forty-fifth day, which approached the normal levels by the end of the experiment. The serum potassium, calcium, and magnesium levels did not show any significant alterations in their concentration.

Avitaminosis A and E. Although the excretion of 11-deoxy-17-oxosteroid did not vary much until about the seventh week, there was a slight subsequent drop in the levels until the terminal stages of the experiment. There were no major changes in the levels of urinary 11-oxy-17-oxosteroids. The average weights of adrenals which showed evidences of hypertrophy increased about 12 per cent over the controls.

The urinary sodium and potassium increased during the terminal week, with the potassium concentration varying more than the sodium. The levels of serum sodium, potassium, magnesium, and calcium ranged within normal limits of the controls.

Discussion. In vitamin A-deficient studies the decrease found in the level of ketosteroids, both the 11-deoxy and 11-oxosteroid fractions and in the weights of adrenal glands indicated that the functional activity of the adrenal cortex was decreased during vitamin A deprivation. These results agree with the hypothesis of Wolf (255) who said that in avitaminosis A the glucocorticoid production would be affected, and that vitamin A-deficiency produces a chemical adrenalectomy, insofar as glucocorticoid production was concerned (258). Although

corticosterone was said to be the principal hormone of the adrenal cortex in rabbits (103), the concentration of this hormone was not measured, since cortisone and cortisol are the principal glucocorticoids whose metabolites form an important fraction of 17-ketosteroids. The present investigations showed that in addition to the corticosterone production being affected by avitaminosis A, cortisone and cortisol production was also decreased. Johnson and Wolf (127) stated that the effect of avitaminosis A on glucorticoid production was not mediated through the adreno-corticotropic hormone. Glyconeogenesis was reported to be suppressed completely in rat when the growth curve reached a plateau.

In the present experiment the deficiency state was not prolonged until the glucocorticoid production was completely supressed. However, it can be observed from the results that not only glucocorticoid production was decreased but also the production of steroid intermediaries like androsterone and dehydroepiandrosterone. It is evident that vitamin A appears to be necessary not only for 11-B-hydroxylation of deoxycorticosterone, as suggested by Johnson and Wolf (127), but also for the metabolism of precusors in the biogenesis of corticosteroid hormones. Although blood glucose levels were not determined in the present investigations, the findings of earlier workers on the effect of avitaminosis A on glucocorticoid synthesis should be confirmed. Since the levels of serum or urinary sodium and potassium were not altered in the deficiency, this provides indirect evidence to show that the mineralocorticoid production

by adrenal cortex remained unaffected.

In vitamin E-deficiency the 11-oxo-steroid production decreased gradually, which indicates a relationship of vitamin E to biosynthesis of adrenal cortical steroids. The investigations of several workers (21, 23, 108) revealed that the adrenal cortex hypertrophies in avitaminosis E. Whether this hypertrophy is due to stress or overactivity or merely an attempt to keep pace with the requirements for hormones could not be clearly elucidated. Vitamin E administration increased the weight of adrenals; the glomerular and fascicular zones showed hyperemia as was reported by Raymondi (203). The effect of vitamin E in this respect was similar to that of adrenocorticotropic hormone. Other workers like Heinsen (105) and Suardi (226) reported increased excretion of ketosteroids after vitamin E had been administered. Whether vitamin E has any direct action on the adrenal cortex in the synthesis of cortical hormones, or whether it operates indirectly through anterior pituitary is not definitely known. Anterior pituitary has been reported by Verzar (245) to have the highest concentration of vitamin E. If such is the case, vitamin E should have some function in the anterior pituitary. In avitaminosis E, a primary hypophyseal disturbance was reported by Ingelman and Sendberg (121). However, their observations were limited to the gonadotropic hormones, which decreased in concentration.

Increased adrenal cortical activity, even before any signs of muscular dystrophy appeared, was visualized by Rosenkrantz (208), who confirmed it by special staining techniques. During

the course of the present investigations it was noticed that the 11-deoxy-17-oxosteroid content of urine increased by about the sixth week, although the 11-oxy-17-oxosteroid fraction continued to decrease. The increase in activity of the adrenal cortex appeared to be limited to only one group of hormones. Furthermore, both fractions of the ketosteroids decreased in their concentrations towards the end. There should have been some changes in glyconeogenesis and in post absorptive blood glucose levels, if there were any deficiencies of glucocorticoid hormones in the body. The present investigations did not study this aspect.

The exact mechanism by which vitamin E enters in the biosynthesis of glucocorticoids, whether indirectly through the anterior pituitary by stimulating the production of adrenocorticotropic hormone or directly on the adrenocortical enzyme systems, is an interesting topic for future investigations.

The weights of the adrenal gland did not alter much except in two cases, which may be due to the fact that only one group of hormones were affected initially. The increase in potassium concentrations with lower sodium content in urine suggests that more potassium is lost from the muscles in muscular dystrophy. The level of mineralocorticoid production was not altered as one may infer from the normal levels of sodium end potassium content in urine and serum. However, the terminal rise of potassium end depression of sodium indicates that the mineralocorticoids were increased.

In contrast to what was observed in the case of avitaminoses

A and E, neither the production of 11-deoxy-17-oxoeteroids nor 11-oxy-17-oxoeteroids showed any change. However, the level of 11-deoxy steroids decreased after the seventh week, and adrenal glands showed evidence of some hypertrophy. The changes in urinary sodium and potassium were comparable to that of pure avitaminosis E, showing an increased concentration of potassium. With the limited data available in these investigations, the interrelationship between vitamin A and E can neither be deduced nor explained, since the results were surprisingly contrary to expectations.

Serum levels of sodium, potassium, calcium, and magnesium in vitamin E-deficiency were in agreement with the findings of Zuckerman and Marquardt (264). They reported no significant alterations in the levels of these cations. But Baldev (7) using lambs, Maplesden and Loosli (147) and Dehority et al. (47) using calves with cod liver oil in the diet, reported decreased serum magnesium concentrations. The latter workers suggested that the depressed magnesium levels might be due to precipitation of magnesium by the large quantity of unsaturated fatty acids present. If this were true, calcium should also have been precipitated, resulting in decreased serum calcium content. The diet employed in the present investigations contained 0.6 per cent linoleic and linolenic acids in addition to what was present in molecularly distilled lard. In spite of such a high quantity of unsaturated fatty acids in the diet, the serum magnesium levels were not altered. This may show that the depression of serum magnesium levels was not due to the high unsaturated fatty acid

content of the diet, but to some factor in cod liver oil which depressed serum magnesium levels by some unknown mechanism. Baldev (7), who studied the serum magnesium levels of dams affected with white muscle disease and their lambs, reported that the magnesium concentration in serum was 0.73 mg. and 0.78 mg. per cent, respectively, whereas the concentrations in healthy dams and their lambs were 1.02 and 1.31 mg., respectively. It appears from the data furnished that even the healthy dams and their lambs had lowered serum magnesium levels. Whether the depression in the affected lambs was significant in the present context is questioned. It may be tentatively concluded that the serum magnesium levels are not affected in avitaminosis E.

The Effect of Avitaminoses A and/or E on the Serum Cholesterol and Ubiquinone Concentration in Tissues

Results. In all groups of rabbits the level of serum cholesterol increased for the first two weeks. This rise might be due to a change in the dietary regimen from a rabbit chow to a semi-purified diet containing comparatively more fat. However, this initial increase did not alter the pattern of subsequent changes as the deficiency progressed. The cholesterol levels in the serum and ubiquinone concentrations in the tissues are presented in Tables 9 and 10, respectively, and the cholesterol levels are graphically represented in Fig. 7.

Vitamin A-Deficiency. The total cholesterol concentration in serum increased during the first 15 days but later on tended to decrease slightly. The free cholesterol content increased

from an initial 23.4 per cent of the total cholesterol to 47.4 per cent in the terminal stages. However, this change was comparable to that in the control group. The serum remained clear in all specimens.

The ubiquinone concentration in skeletal muscles, liver, and adrenal gland was higher than in the control group.

Vitamin E-Deficiency. The total cholesterol content increased markedly in both the free and esterified cholesterol. The free cholesterol content of the total cholesterol, however, rose remarkably from an initial 23.4 per cent to 58.04 per cent over a 60-day period. Another important observation in this connection was the milky or turbid appearance of the serum in all the animals of this group, particularly so after the animals were on the diet for 30 days. The levels of free as well as total cholesterols demonstrated a gradual rise from the beginning towards the end of the experiment.

The ubiquinone content of skeletal muscle and liver decreased, while that of adrenal gland increased.

Vitamins A and E Deficiency. The serum cholesterol levels were elevated, although not to the same extent as that of vitamin E-deficient animals. The cholesterol levels were intermediary between those of vitamin A-deficient and vitamin E-deficient groups. The free cholesterol content rose from an initial 20 per cent of total cholesterol to 62.8 per cent during the 60-day experimental period.

The ubiquinone concentrations in skeletal muscle, adrenal cortex, and liver were decreased considerably and were even less

than in avitaminosis E.

During the terminal stages the serum in one animal was slightly turbid but never milky in appearance.

Discussion. The findings of the present investigations on vitamin A-deficiency agree well with those reported by Phillips (189) in rats. No data on rabbits were available in the literature. Total and free cholesterol levels did not decrease considerably, probably because the deficiency state was in the early stages at the time the experiment was terminated. These observations suggest that vitamin A-deficiency had slight affect, if any, on the synthesis of cholesterol. Since the ubiquinone content of all the tissues studied was increased, the hypothesis of Phillips (189) that suppression of cholesterol synthesis results in an increased accumulation of squalene, which in turn forces the reaction towards the synthesis of ubiquinone, is supported by the present findings.

In avitaminosis E the serum cholesterol, more particularly the free cholesterol content, increased; these findings are quite in accordance with the observations of Deuel et al. (48). That the rise in cholesterol content precedes the muscular dystrophy signs as observed by Shull et al. (219) also agrees with the present observations, wherein the serum cholesterol level rose by the thirtieth day, and signs of dystrophy appeared after the fifth or sixth week on the vitamin E-deficient diet. The milky or turbid appearance of the serum may be due to an increased quantity of lipoproteins. The same phenomena were reported by Oppenheimer and Millman (181) in rabbits and by

Table 9. Total and free cholesterol concentrations[1] in the serum at 15-day intervals.

Group	Days				
	0	15	30	45	60
A deprived					
Total cholesterol	58.2	152.7	129.5	131.8	140.0
Free cholesterol	13.6	52.7	100.0	88.6	66.6
E deprived					
Total cholesterol	61.8	120.0	143.2	272.7	342.2
Free cholesterol	14.5	47.3	110.2	181.8	198.5
A and E deprived					
Total cholesterol	54.5	109.1	180.0	181.8	269.0
Free cholesterol	10.9	29.1	136.4	140.0	174.8
Control					
Total cholesterol	54.5	120.0	90.0	79.6	88.4
Free cholesterol	16.3	32.7	31.2	43.2	41.3

[1]Milligrams per 100 ml. of serum.

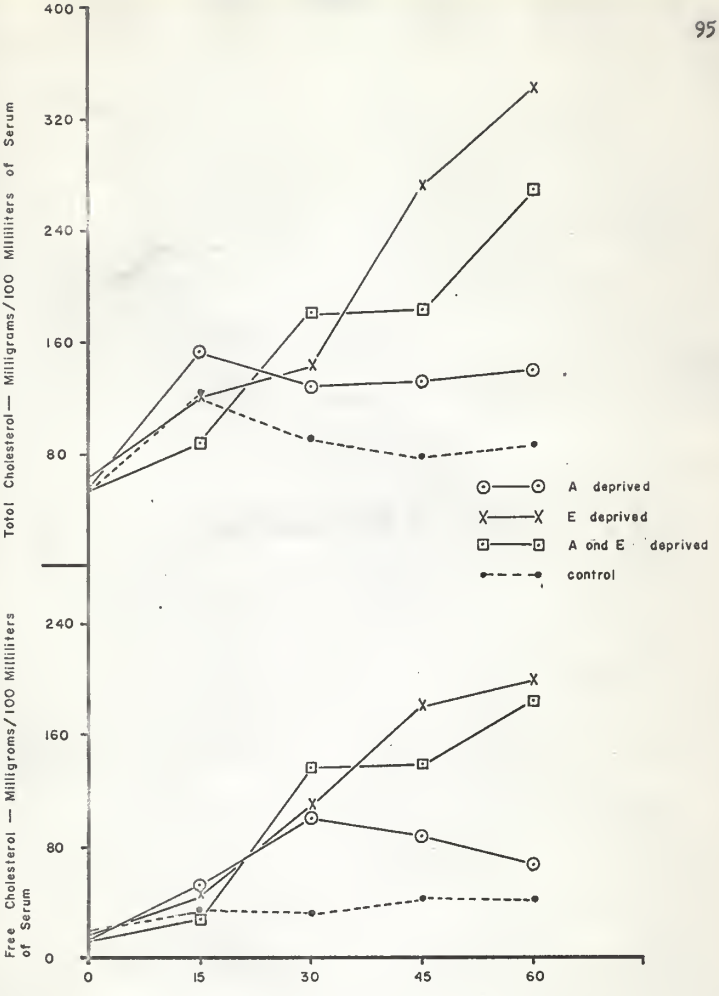

Fig. VII. Variation in free and total cholesterol in serum of vitamins A and/or E deprived rabbits as compared to controls at 15 day intervals.

Table 10. Ubiquinone[1] content of skeletal muscle, liver, and adrenal glands in avitaminoses A and/or E in rabbits.

Group	Number of animals	Ubiquinone[1]		
		Skeletal muscle	Liver	Adrenal
A deprived	4	233.42	286.1	2417.1
E deprived	3	148.2	162.2	2322.5
A and E deprived	4	143.4	160.7	722.2
Control	3	182.2	219.7	901.2

[1]Micrograms/gram of fresh tissue.

Baldev (7) in lambs.

The concentration of ubiquinone in the liver and skeletal muscle was decreased, while the concentration in adrenal gland was increased. These observations on liver are in agreement with those of Edwin et al. (66) and Diplock et al. (60) in rats. Ubiquinone levels in tissues were increased when either alpha-tocopherol or selenium was administered to deficient rats, poults, or chickens (61). Phillips (189) observed that the ubiquinone levels were related to the vitamin E status of the animal, although Lee et al. (141) disagreed on this point. Ubichromenol, a possible metabolite of ubiquinone, whose concentration is increased in tissues deficient in vitamin E, was shown to partly modify the effects of avitaminosis E by decreasing the incidence of encephalomalacia in chicks (210) or preventing the resorption of fetuses in rats (128). The exact relationship between vitamin E, selenium, ubichromenol, and ubiquinone is as yet unknown. Vitamin E probably affects the biosynthesis of ubiquinone, which has been conclusively shown to be an integral part of electron transport chain. Tocopherol by itself is thought to be concerned with biological oxidations. It is quite possible that the activity of tocopherol in biological oxidations may be mediated indirectly through ubiquinone.

In avitaminoses A and E the levels of cholesterol in serum were intermediate between levels in avitaminosis A and avitaminosis E groups. However, the ubiquinone levels were considerably below that of vitamin A or E-deficient groups. These observations suggest a possible interrelationship between vitamins

A and E in cholesterol and ubiquinone biosynthesis. It is also apparent from these studies that there seems to be a biological antagonism between these two vitamins. The presence of both vitamins appears to maintain a normal balance between the levels of ubiquinone and cholesterol in the tissues. Vitamin A probably suppresses the production of ubiquinone but favors the synthesis of cholesterol, whereas vitamin E has the opposite effect. In vitamin E-deficiency the serum cholesterol increased since vitamin A, which was present in the tissues, favors cholesterol synthesis. In avitaminosis the utilization of cholesterol may also be decreased. These observations also suggest a possible role of vitamin E in lipid metabolism. Since the plasma cholesterol levels were higher with increased lipoprotein content in serum, a relationship between predisposition for atheromatous conditions and vitamin E-deprivation may be presumed.

The disagreeability of various investigators regarding the levels of ubiquinone in avitaminosis E may be due to the different levels of vitamin A administered.

The Effect of Avitaminosis A and/or E on the Changes in Cellular Constituents of Blood

The packed cell volumes, total leucocyte count, and differential leucocyte count of blood collected from the ear veins of each group at weekly intervals is presented in Table 11.

Results. There were no significant changes, except for normal fluctuations that would be expected, in the packed cell volumes in any group of animals. In all the groups except the

Table 11. Packed cell volume, total leucocyte, and differential leucocyte counts at weekly intervals.[1]

Group	Weeks								
	0	1	2	3	4	5	6	7	8
A deprived									
Packed cell volume	45.6	42.8	39.75	43.80	40.70	42.20	37.10	41.60	-
Total leucocyte count	9,313	10,596	10.967	9,623	9,258	12,603	13,362	13,797	-
% Neutrophiles (Segmented)	11.50	19.80	11.40	17.75	18.40	12.87	19.17	23.62	-
% Juvenile	0.00	0.50	0.450	0.25	2.00	0.12	0.33	0.33	-
% Band	0.00	1.37	0.58	1.25	1.85	0.43	0.50	1.17	-
% Esoinophiles	1.45	0.75	0.95	2.00	0.38	0.51	0.00	0.67	-
% Monocytes	1.85	1.37	1.70	2.60	2.17	3.15	0.83	1.62	-
% Basophiles	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
% Lymphocytes	84.75	76.25	85.25	76.25	75.75	82.83	79.17	70.80	-
E deprived									
Packed cell volume	40.80	40.95	41.40	39.40	42.90	43.30	41.40	44.10	
Total leucocyte count	9,086	22,842	11,860	14,093	10,433	19,157	17,097	29,056	12,980
% Segmented	19.50	17.20	13.75	26.95	24.37	22.70	27.17	33.83	35.70
% Juvenile	0.75	0.25	0.00	0.38	0.25	0.83	0.00	0.00	0.33
% Band	1.12	0.5	1.00	0.92	1.12	0.83	1.33	1.20	0.67
% Eosinophiles	1.00	1.00	0.38	3.12	2.50	0.00	0.00	0.00	0.00
% Monocytes	2.00	1.62	1.25	2.25	1.12	3.00	1.17	3.00	3.67
% Basophiles	0.37	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00
% Lymphocytes	75.20	79.40	81.10	68.90	75.62	72.70	70.33	62.00	59.67

[1]Determination made once a week.

Table 11 (Concl.). Packed cell volume, total leucocyte, and differential leucocyte counts at weekly intervals.

Group	Weeks 0	1	2	3	4	5	6	7	8
A and E deprived									
Packed cell volume	47.3	48.5	39.8	42.7	41.2	45.4	37.8	48.0	-
Total leucocyte count	13,003	23,144	13,740	17,399	7,757	27,053	27,559	-	-
% Neutrophiles (Segmented)	27.88	39.33	15.03	21.25	26.38	21.75	27.62	46.00	-
% Juvenile	0.62	0.78	0.00	0.00	0.50	0.12	0.00	0.00	-
% Band	1.00	0.62	0.25	0.75	0.88	1.00	0.75	2.00	-
% Eosinophiles	1.00	1.28	1.12	0.50	1.25	0.12	0.00	1.00	-
% Monocytes	2.83	0.88	1.12	1.75	2.15	2.00	1.25	3.00	-
% Basophiles	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	-
% Lymphocytes	67.38	66.50	82.15	75.5	68.88	70.00	70.50	48.00	-
Control									
Packed cell volume	44.6	45.4	38.9	40.3	38.8	41.7	33.3	28.2	-
Total leucocyte count	11,488	8,342	10,914	6,808	9,132	10,892	12,450	18,177	-
% Segmented	44.17	27.82	14.83	24.83	19.67	20.17	20.50	12.50	-
% Juvenile	1.00	0.08	0.00	0.50	0.17	0.17	0.00	0.00	-
% Band	0.00	1.43	1.00	0.50	0.83	0.50	1.50	1.00	-
% Eosinophiles	1.17	1.67	0.83	1.67	0.67	0.00	0.50	1.00	-
% Monocytes	2.50	2.80	2.67	3.67	2.67	1.83	2.50	1.50	-
% Basophiles	0.67	0.00	0.00	0.67	0.17	0.00	0.00	0.00	-
% Lymphocytes	49.83	68.05	80.67	69.17	75.83	77.3	75.00	81.00	-

control group the total leucocyte count and the percentage of neutrophile leucocytes increased, whereas the lymphocytes decreased as the deficiency state progressed. There was little, if any, change in the eosinophils except in vitamin E-deficient animals where no eosinophils could be found during the last four weeks. The increase in total leucocyte count was not great enough to be called an absolute leucocytosis.

Discussion. A perusal of the packed cell values indicated that erythropoiesis was not affected in any of the deficiency states. The hemoglobin concentration taken during terminal phases of the experiment did not reveal any decrease in vitamin A-deficiency. In rats a decrease in hemoglobin or erythrocytes was reported in avitaminosis A by Sure et al. (233) and Frank (80). However, Frank (80) also reported a decrease in leucocyte count with an increase in ratio of neutrophiles to lymphocytes in rats. The present observations agree well in regards to the change in the leucocyte picture but differ in the total leucocyte count. Changes in leucocyte and differential leucocyte counts, however, were not high enough to be called leucocytosis.

In avitaminosis E the changes observed in rabbits were consistent with what was reported in calves by Maplesden and Loosli (147).

Dinning and Day (57) described in monkeys an anemia with reduced erythrocyte count due to inadequate erythropoiesis which might be due to lack of a specific maturation factor. In a review on vitamin E responsive macrocytic anemias in children, Majaj et al. (146) reported that vitamin E was effective in the

treatment of certain macrocytic anemias in human infants under treatment for marasmus and kwashiorkar. The above reports suggest a role for vitamin E in erythropoiesis. However, in animals other than monkeys, depression of erythropoiesis was not reported. During the course of the present investigations, hemoglobin levels were determined at random, and it was observed that one animal in an E-deficient group and two animals in A and E-deficient groups demonstrated a decreased hemoglobin concentration during the last two weeks of the experiment. A detailed study of the deficient groups at the terminal stages may throw some light in this connection. The cause of such an anemia cannot be adequately explained at present. The investigations of Greenberg et al. (82) have shown a reletionship between ascorbic acid and vitamin E in the absorption of iron. Further investigations are needed before any definitive conclusions can be drawn from the meager data available at present.

Effect of Avitaminoses A and/or E on the Oxygen Consumption of Skeletal Muscle Strips and the Effect of in vitro Addition of Alpha-tocopherol and/or Sodium Selenite to the Incubation Medium on Tissue Respiration

Results. The oxygen consumption of skeletal muscle strips taken from the semitendinosus muscle was measured in vitro in the various groups of rabbits. The effect of addition of alpha-tocopherol acetate, 5 mg. per cent, emulsified in the Krebs' Ringer phosphate buffer with the help of bile salts, selenium as sodium selenite at a concentration of 0.10 part per million, and a combination of both on the oxygen uptake by the skeletal

muscle strips was investigated. The data are presented in Table 12. The slices of cardiac muscle were taken at the apex, which was fibrosed due to repeated cardiac puncture. The results were inconsistent and, hence, are not included.

In the control group the oxygen consumption by the skeletal muscle was decreased by about 22.4 per cent with the addition of sodium selenite to the medium. However, addition of vitamin E did not alter the oxygen uptake to any significant extent. A combination of both the substances too, surprisingly, did not elicit any change in tissue respiration in the control animals.

The oxygen uptake was not apparently altered either by a deficiency of vitamin A or by the addition of alpha-tocopherol and/or selenite to the medium containing the tissues. The tissues obtained from avitaminosis E muscle appear to show a significant increase of 237 per cent in oxygen consumption over the normal control group. Addition of sodium selenite decreased the oxygen uptake in this group by 65.6 per cent, while alpha-tocopherol depressed the oxygen uptake by 25 per cent, and a combination of both substances lowered oxygen uptake by only 55.3 per cent. Although the above data were calculated over a group average under each treatment, overlapping individual values were obtained. Two animals in the vitamin E-deficient group, E-3 and E-4, had an increase of only 60 per cent. From the limited data, only tentative conclusions concerning alterations in oxygen uptake may be made. In the vitamins A and E-deficient group the values were not apparently different from those of controls or the vitamin A-deficient group. The addition

Table 12. The effect of *in vitro* addition of alpha-tocopherol and/or sodium selenite on average oxygen consumption[1] of skeletal muscle strips.

Group and animal number	Per cent dry matter	Oxygen consumption[1]			
		Krebs' Ringer solution	Krebs' Ringer solution with selenite	Krebs' Ringer solution with vitamin E	Krebs' Ringer solution with vitamin E and sodium selenite
Group A					
A-1	16.05	1.43	0.77	1.61	1.64
A-2	16.84	0.80	0.84	1.08	1.61
A-3	16.53	1.80	1.67	1.32	2.29
Average	16.47	1.68	1.64	1.30	1.85
Group E					
E-1	19.03	4.06	1.33	2.08	0.88
E-2	20.77	2.22	0.38	1.25	0.06
E-3	13.17	1.65	1.60	1.43	0.45
E-4	16.33	1.85	0.85	1.25	2.52
Average	17.32	2.44	0.84	1.83	1.19
Group AE					
AE-1	15.8	1.32	2.02	2.21	4.49
AE-3	15.6	1.09	2.24	1.36	1.25
AE-4	16.8	2.24	1.16	1.66	1.82
Average	16.07	1.55	1.81	1.74	2.62
Control					
C-2	15.4	1.35	0.88	1.27	1.39
C-3	15.2	0.63	0.71	0.65	0.91
C-4	16.2	1.12	0.81	1.22	1.08
Average	15.6	1.03	0.80	1.05	1.13

[1]Microliters per milligram of dry tissue per hour.

of alpha-tocopherol or sodium selenite or a combination of both did not show any apparent trend or change in the oxygen consumption.

Discussion. Several investigators reported that the oxygen consumption in vitamin E-deficient tissues was increased by 200 to 300 per cent. That the injection of alpha-tocopherol a few hours prior to sacrifice of the animal, or addition of water soluble tocopherol phosphate *in vitro* to the incubation medium reduced the oxygen uptake was demonstrated by Houchin (112) and Houchin and Mattil (114). However, the latter workers reported that tocopheryl acetate added in an emulsified form did not reduce the oxygen uptake by the dystrophic muscle. The normal muscle respiration wss not affected by the *in vivo* or *in vitro* addition of tocopherol in any form. The reason for the ineffectiveness of tocopheryl acetate in an emulsified state may be due to the fact that it could not cross the cell barrier and reach the site for its activity. McLean (154), who studied the changes in ion transport in normal and vitamin E-deficient tissues, reported that the liver slices lost their ability to reaccumulate potassium removed by leaching the tissues in cold, and as a result the subsequent oxygen uptake was lowered. The differences reported by several investigators on the oxygen consumption of tissues may be due to the varied techniques used in preparing the tissues. *In vivo* injection of selenium compounds to vitamin E-deficient animals was reported to restore cell respiration to normal. The role of selenium, if any, in the utilization of alpha-tocopherol acetste emulsion was studied.

Since chilling the tissues was reported to result in irreversible changes in ion transport of the vitamin E-deficient tissues, attempts were made to keep the muscle strips at 37°C. from the time they were removed from the animal. Although Rosenkrantz (207) demonstrated that addition of calcium salts to the incubation medium reduced the oxygen uptake of the tissues, it was felt that it would be better to simulate the normal environment of the tissues as much as possible while studying their activity in vitro. With the modifications described above the present investigations were carried out.

There were no data available regarding the oxygen uptake of tissues in a superimposed deficiency of vitamin A on avitaminosis E.

The results of the present investigations reveal that selenium depresses the oxygen uptake of normal as well as vitamin E-deficient tissues. However, the effect on tissues from avitaminosis A and avitaminosis A and E was not clear. Potter and Elvehjem (195) reported that selenium inhibits oxygen uptake by tissues in vitro which appeared to be mediated through poisoning of succinic dehydrogenase. Selenium has now been included in the list of essential elements. Concentration of 0.10 parts per million should have suppressed succinoxidase activity which instead increased two or threefold in avitaminosis E (113, 115). Vitamin E decreases the succinoxidase activity in vitamin E-deficient tissues. The activities of these substances may be complementary to each other. Addition of alpha-tocopherol decreased the oxygen consumption of the muscle strip in vitro,

though not to the same extent as selenium. A combination of these substances reduced the tissue respiration. It can be tentatively concluded that selenium is nonspecific in reducing succinoxidase activity since it reduces the oxygen uptake of even normal tissues, and that the action of alpha-tocopherol is specific, its activity being restricted to only deficient tissues.

It is surprising to note that neither selenium nor alpha-tocopherol affected the muscle tissue respiration in avitaminosis A, and vitamin A-deficiency superimposed with E. Whether the presence of vitamin A is required for the *in vitro* activity of alpha-tocopherol in influencing cellular activity is a problem for future investigations. However, no data is available to show the effect of administration of vitamin E or selenium to either vitamin A-deficiency or A and E combined deficiencies. Since the present investigations were limited, no definite conclusions can be drawn from the data obtained.

SUMMARY

Employing sixteen New Zealand white rabbits, the role of vitamins A and E in the biosynthesis of hormones of the adrenal cortex, the relationship between vitamin A and E in the synthesis of ubiquinone, and the effect of selenium and vitamin E on *in vitro* cellular respiration were studied.

Levels of glutamic oxalacetic transaminases in serum rose earlier than creatine and gave an earlier indication of the deficiency status. The serum glutamic pyruvic transaminase levels

increased steadily from the fifteenth day on, indicating some latent damage to either the liver or kidney, which was not apparent at post mortem examination. The levels of glutamic pyruvic transaminase in serum were not affected in vitamin A-deficiency; while in the combined deficiency of vitamins A and E, the levels were 16 times more than the basal levels and about eight times more than the controls animals during the same period. The liver presented a parboiled and mottled appearance at post mortem examination in three animals.

The urinary 11-oxy-17-oxo-steroids decreased gradually in vitamins A or E-deficiencies, while the 11-deoxy-17-oxo-steroids decreased in vitamin A-deficiency but not in vitamin E-deficiency. In avitaminosis A and E the changes did not show any significant trend. The production of adrenal glucocorticoids appeared related to vitamins A and E. The sodium and potassium concentration in urine and serum, which was not altered significantly provides indirect proof that the mineralo-corticoid production was not affected by these deficiencies. Neither the serum nor the calcium levels changed significantly in the present investigations.

In avitaminosis E the serum cholesterol levels were elevated 20 times at 30 days, even before the symptoms of muscular dystrophy were apparent. Free cholesterol levels increased much more than the esterified cholesterol in the serum. In vitamin A-deficiency the levels of cholesterol showed a slight tendency to decrease after an initial rise, the free cholesterol decreasing more than the total concentration. In the group deficient in

both vitamins A and E the levels of free and total cholesterol were intermediate between the levels of vitamin A and vitamin E-deficient groups. The ubiquinone content of liver and muscle was decreased in avitaminosis E, lowered considerably in liver, skeletal muscle, and adrenal gland in avitaminosis A and E, but increased in vitamin A-deficiency in all the tissues studied. There appears to be biological antagonism between vitamins A and E in maintaining normal levels of cholesterol and ubiquinone in the body.

The oxygen uptake of avitaminosis E tissues in vitro was increased by 237 per cent. Addition of sodium selenite to the medium depressed the oxygen uptake of the control group by 22.4 per cent and that of vitamin E-deficient by 65.6 per cent, but had no significant effect on the vitamin A-deficient and A and E-deficient groups. Emulsified alpha-tocopherol decreased the oxygen consumption of the skeletal muscle by 25 per cent in the vitamin E-deficient group and none in the other groups. The effect of selenium appeared to be nonspecific, since it also decreased the tissue respiration in control animals. However, the reasons for the ineffectiveness of either selenium or alpha-tocopherol to alter the oxygen uptake of tissues from vitamin A and E-deficiency and the ineffectiveness of selenium in vitamin A-deficient tissues cannot be properly explained from the limited data.

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AVITAMINOSES A AND/OR E RABBITS ON SEMI-PURIFIED DIET: ALTERATIONS IN BLOOD, URINE, TISSUE CONSTITUENTS, AND CELLULAR ACTIVITY

by

KONGARA SATYANARAYANA RAO

B. V. Sc. Madras University (India), 1945

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Employing sixteen New Zealand white rabbits, the investigatione were carried out to etudy the biological role of vitamine A and E in the synthesis of hormones of the adrenal cortex and ubiquinone, and the effect of selenium and vitamin E on the oxygen uptake of skeletal muecle tiesue in vitro.

In avitaminosis A there wae no increase in either urinary creatine or serum transaminasee. In vitamin E or A and E-deficiency the level of serum glutamic oxalacetic transaminaee (SGOT) wae elevated earlier than the increased creatine/creatinine ratio, giving an earlier indication of muscular dystrophy. The eerum glutamic pyruvic transaminase (SGPT) level increased steadily from the fifteenth day, which indicated eome latent damage to either the liver or kidney, not apparently noticed at post mortem examination. In the combined deficienciee of vitamins A and E the SGPT levele were coneiderably higher, and in this group the liver in moet animals presented either a parboiled or mottled appearance.

The excretion of 11-oxy-17-oxosteroids decreased gradually in the groupe deprived of vitamins A or E, while the 11-deoxy fraction decreased only in the vitamin A deprived group. In avitaminoeis A and E, no particular recognizable trend wae noticed. That neither the urinary nor eerum sodium and potaseium concentrations were altered to any significant extent in any of the groupe indicates that the mineralocorticoid elaboration wae not altered. The serum calcium and magnesium levele were within normal range in all the groups.

The serum cholesterol levels were elevated during the first

five days in all groups due to s chsnge to the semi-purified diet. Subsequently the free as well ss the totsl cholesterol concentrations were increased in E or A snd E-deficient groups, although a little less in the lstter. In the A-deficient group the cholesterol level was not affected to sny significsnt extent. The ubiquinone content of the liver, skeletsl muscle, snd adrensl wss higher in A-deficient snd lower in the E or A snd E-deficient groups.

In all the deficient groups the cellulsr constituents of blood revesled s mild leucocytosis with slight neutrophilis and lymphopenia.

The studies on oxygen uptake in vitro showed an increase in vitsmin E-deficient tissue which wss decressed by adding either vitamin E snd/or selenium to the medium. Selenium depressed the oxygen uptske of the control group, wheress it hsd no effect on the A or A snd E-doficient groups.